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(54) Title: COMBINATORIAL SYNTHESIS OF MULTIBINDING LIBRARIES			
(57) Abstract Disclosed are methods for synthesizing large collections of diverse multimeric compounds as well as iterative processes for evaluating key molecular constraints imparting multibinding properties to multimeric compounds. Also disclosed are libraries of multimeric compounds which can be evaluated for imparting multibinding properties.			

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COMBINATORIAL SYNTHESIS OF MULTIBINDING LIBRARIES

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the following U.S. Provisional Applications:

1. U.S. Provisional Application No. 60/088,466, filed June 8, 1998;
 - 10 2. U.S. Provisional Application No. 60/088,464, filed June 8, 1998;
 3. U.S. Provisional Application No. 60/088,465, filed June 8, 1998;
 4. U.S. Provisional Application No. 60/088,448, filed June 8, 1998;
 5. U.S. Provisional Application No. 60/092,938, filed July 15, 1998;
 6. U.S. Provisional Application No. 60/092,941 filed July 15, 1998;
 - 15 7. U.S. Provisional Application No. 60/093,068, filed July 16, 1998;
 8. U.S. Provisional Application No. 60/093,072, filed July 16, 1998;
- Each of these applications are incorporated herein by reference in its entirety.

20

BACKGROUND OF THE INVENTION

Field of the Invention

This invention is directed to methods for synthesizing very large collections of diverse multimeric compounds. Such collections or libraries of compounds permit facile assessment of which multimeric ligand compounds possess multibinding properties.

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This invention also is directed to methods for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor.

30

This invention is further directed to libraries of such diverse multimeric derivatives.

References

The following publications, patents and patent applications are cited in this application as superscript numbers:

- 5 ¹ Gallop, et al., *U.S. Patent No. 5,525,734*, for "Methods For Synthesizing Diverse Collections of Pyrrolidine Compounds", issued June 11, 1996
- ² Gallop, et al., International Patent Application Publication No. WO 96/16333 for "Methods for Synthesizing Diverse Collections of β -lactam Compounds", published 30 May 1996
- 10 ³ Costa, et al., *Chemical Pharmacology*, **34**(1):25-30 (1985)
- ⁴ Pathare, et al., *Bioconjugate Chem.*, **8**(2):161-170 (1997)
- 15 ⁵ Pitha, et al., *J. Med. Chem.*, **26**(1):7-10 (1983)
- ⁶ Skorobogaty, et al., *Anti-Cancer Drug Design*, **3**:41-56 (1988)
- 20 ⁷ Priebe, et al., International Patent Application Publication No. WO 97/34612, for "Bis-Anthracyclines with High Affinity Against Doxorubicin Resistant Tumors", published September 25, 1997
- ⁸ Fesik, et al., *U.S. Patent No. 5,891,643*, for "Use of Nuclear Magnetic Resonance to Design Ligands to Target Biomolecules", issued June 11, 1996
- 25 ⁹ International Patent Application Publication No. WO 93/06121.
- 30 ¹⁰ Pirrung, et al., "Large Scale Photolithographic Solid Phase Synthesis of Polypeptides and Receptor Binding Screening Thereof", U.S. Patent No. 5,143,854, issued September 1, 1992.

 All of the above publications, patents and patent applications are herein

35 incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

40 Compounds having biological/pharmaceutical activity can be identified by screening individual compounds or diverse collections of compounds (i.e., libraries of

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compounds) produced through either molecular biological or synthetic chemical techniques. The use of libraries of such compounds has been emphasized in the past few years as a research tool primarily because such libraries stream-line drug discovery.

5 Heretofore, such libraries have been primarily focused on the synthesis of diverse collections on monomeric ligands targeting a single receptor or a group of receptors. See, for example, Gallop, et al.^{1,2} This is consistent with the pharmaceutical industry's focus on monomeric ligands as drug therapies for a variety of disease conditions.

10 Recently, there have been theoretical discussions by, for example, Costa, et al.³ and others concerning possible enhancements in activity arising from dimeric and higher multimeric compounds. Such enhancements were contemplated as arising from potential multibinding properties imparted by these compounds. These multibinding properties include, by way of example, one or more of increased affinity, increased selectivity for
15 target, increased specificity for target, increased potency, increased efficacy, decreased toxicity, improved duration of activity or action, increased ability to kill cells such as fungal pathogens, cancer cells, etc., decreased side effects, increased therapeutic index, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like.

20 Notwithstanding these theoretical benefits associated with multibinding compounds, not all dimer or oligomeric compounds exhibit multibinding properties and, in fact, the art is replete with examples of dimers and multimers exhibiting diminished properties.^{4,5,6} In fact, references providing rationalized approaches for synthesizing
25 dimeric compounds required determination of the X-ray crystal structure of the monomeric ligand/target complexes as disclosed by Priebe, et al.⁶ or of the NMR spectrum of ligand/target complexes as disclosed by Fesik, et al.⁷ Extrapolation from the structural information so obtained allowed for a rational basis for synthesizing individual dimers. However, as is apparent, this approach is tedious at best and, nevertheless,
30 approaches the synthesis of candidate multibinding compounds on a compound-by-compound basis.

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In view of the above, there is an ongoing need to develop techniques wherein a large collection of multimeric compounds which are candidates for possessing multibinding properties can be prepared and then tested for the presence of multibinding properties. Such techniques would permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. These molecular constraints could then be used in further iterations of the process to provide further definition of key molecular constraints required to impart multibinding properties for the multimeric compounds.

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SUMMARY OF THE INVENTION

This invention is directed to general synthetic methods for generating large libraries or collections of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. In one embodiment, the general synthetic methods employ combinatorial aspects to provide for libraries of multimeric compounds which compounds can then be assayed for multibinding properties. In another embodiment, a collection of multimeric compounds is prepared and processed through an iterative process to determine those molecular constraints necessary to impart multibinding properties.

20

In the library aspect, the diverse multimeric compound libraries provided by this invention are synthesized by combining a linker or linkers (i.e., a library of linkers) with a ligand or ligands (i.e., a library of ligands) to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity, polarization and polarizability. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

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This invention is also directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a
5 ligand or a class of ligands targeting a receptor.

This invention is still further directed to iterative methods to determine those molecular constraints necessary to impart multibinding properties.

10 Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

- (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;
- 15 (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the
20 library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and
- (d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

25

In another of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

- (a) identifying a library of ligands wherein each ligand contains at least one
30 reactive functionality;

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(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition of ligands is preferred when a mixture of different ligands is employed to ensure that heterodimeric or multimeric compounds are prepared. Concurrent addition of the ligands is preferred when it is desired that at least a portion of the to-be-prepared multimeric compounds will be homomultimeric compounds.

The assay protocols recited in (d) can be conducted on the multimeric ligand compound library produced in (c) above, or preferably, each member of the library can first be isolated, for example, by preparative liquid chromatography mass spectrometry (LCMS) and then assayed.

In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

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(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

5 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

10 In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

15 (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or
20 mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In a preferred embodiment, the library of linkers employed in either the methods
25 or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability, and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having
30 different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

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In another preferred embodiment, the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, boronates, anhydrides, and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heteromeric (i.e., at least one of the ligands is different from the other ligands).

In addition to the combinatorial methods described herein, this invention provides for an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

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(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c)
5 above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding
10 properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

15 Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

20 BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates examples of multibinding compounds comprising 2 ligands attached in different formats to a linker.

FIG. 2 illustrates examples of multibinding compounds comprising 3 ligands
25 attached in different formats to a linker.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, this invention provides methods for synthesizing large
30 collections of diverse multimeric compounds as well as iterative processes for evaluating key molecular constraints imparting multibinding properties to multimeric compounds.

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In addition, this invention provides for libraries of multimeric compounds which can be evaluated for imparting multibinding properties. When discussing such methods or libraries, the following terms have the following meanings unless otherwise indicated. Any undefined terms have their art recognized meanings.

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The term "library" refers to at least 3, preferably from 10^2 to 10^9 and more preferably from 10^2 to 10^4 multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric
10 compounds can be directly assayed for multibinding properties. In another embodiment, each member of the library of multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

The term "collection" refers to a set of multimeric compounds which are prepared
15 either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to 10^9 members and still more preferably from 10 to 10^4 members.

The term "multimeric compound" refers to compounds comprising from 2 to 10
20 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from 1 to 40 carbon atoms, more preferably 1 to 10
25 carbon atoms, and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, *n*-decyl, tetradecyl, and the like.

The term "substituted alkyl" refers to an alkyl group as defined above, having
30 from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl,

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substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene (-CH₂-), ethylene (-CH₂CH₂-), the propylene isomers (e.g., -CH₂CH₂CH₂- and -CH(CH₃)CH₂-) and the like.

The term "substituted alkylene" refers to an alkylene group, as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such fused groups contain from 1 to 3 fused ring structures.

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The term "alkaryl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

5 The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

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The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

15

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of
20 example, methylenemethoxy (-CH₂OCH₃), ethylenemethoxy (-CH₂CH₂OCH₃), *n*-propylene-*iso*-propoxy (-CH₂CH₂CH₂OCH(CH₃)₂), methylene-*t*-butoxy (-CH₂-O-C(CH₃)₃) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of
25 example, methylenethiomethoxy (-CH₂SCH₃), ethylenethiomethoxy (-CH₂CH₂SCH₃), *n*-propylene-*iso*-thiopropoxy (-CH₂CH₂CH₂SCH(CH₃)₂), methylene-*t*-thiobutoxy
30 (-CH₂SC(CH₃)₃) and the like.

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The term "alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. Preferred alkenyl groups include ethenyl ($-\text{CH}=\text{CH}_2$), *n*-propenyl ($-\text{CH}_2\text{CH}=\text{CH}_2$), *iso*-propenyl ($-\text{C}(\text{CH}_3)=\text{CH}_2$), and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, $-\text{SO}-\text{alkyl}$, $-\text{SO}-\text{substituted alkyl}$, $-\text{SO}-\text{aryl}$, $-\text{SO}-\text{heteroaryl}$, $-\text{SO}_2-\text{alkyl}$, $-\text{SO}_2-\text{substituted alkyl}$, $-\text{SO}_2-\text{aryl}$ and $-\text{SO}_2-\text{heteroaryl}$.

The term "alkenylene" refers to a diradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. This term is exemplified by groups such as ethenylene ($-\text{CH}=\text{CH}-$), the propenylene isomers (e.g., $-\text{CH}_2\text{CH}=\text{CH}-$ and $-\text{C}(\text{CH}_3)=\text{CH}-$) and the like.

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The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, and preferably from 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy,

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thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

The term "alkynyl" refers to a monoradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 20 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynyl groups include ethynyl (-C≡CH), propargyl (-CH₂C≡CH) and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

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The term "alkynylene" refers to a diradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynylene groups include ethynylene (-C≡C-), propargylene (-CH₂C≡C-) and the like.

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The term "substituted alkynylene" refers to an alkynylene group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

The term "acyl" refers to the groups HC(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acylamino" or "aminocarbonyl" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" or "alkoxycarbonylamino" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

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The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

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The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

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Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

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The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

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The term "arylene" refers to the diradical derived from aryl (including substituted aryl) as defined above and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group -NH₂.

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The term "substituted amino" refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" or "alkoxycarbonyl" refers to the groups "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "cycloalkylene" refers to the diradical derived from cycloalkyl as defined above and is exemplified by 1,1-cyclopropylene, 1,2-cyclobutylene, 1,4-cyclohexylene and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl,

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-SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

5 The term "substituted cycloalkylene" refers to the diradical derived from substituted cycloalkyl as defined above.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

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The term "cycloalkenylene" refers to the diradical derived from cycloalkenyl as defined above and is exemplified by 1,2-cyclobut-1-enylene, 1,4-cyclohex-2-enylene and the like.

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The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

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The term "substituted cycloalkenylene" refers to the diradical derived from substituted cycloalkenyl as defined above.

30 The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

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The term "pseudohalide" refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups include, by way of example, mesyl, tosyl, azido and cyano groups.

5 The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

10 Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, 15 carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy. 20 Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

25 The term "heteroaryloxy" refers to the group heteroaryl-O-.

 The term "heteroarylene" refers to the diradical group derived from heteroaryl (including substituted heteroaryl), as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylenes, 1,8-quinolinylenes, 1,4-benzofuranylene, 2,5-pyridnylene, 2,5-indolenyl and the like.

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The term "heterocycle" or "heterocyclic" refers to a monoradical saturated unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

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Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

The term "heterocycloxy" refers to the group heterocyclic-O-.

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The term "thioheterocycloxy" refers to the group heterocyclic-S-.

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The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

5 The term "oxyacylamino" or "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

10 The term "spiro-attached cycloalkyl group" refers to a cycloalkyl group attached to another ring via one carbon atom common to both rings.

The term "thiol" refers to the group -SH.

15 The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

20 The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

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As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

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The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds (including intermediates thereof) prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzyldine, phenacyl, t-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable thiol blocking groups include disulfide groups, acyl groups, benzyl groups, and the like.

Preferred removable amino blocking groups include conventional substituents such as t-butyloxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (Fmoc), allyloxycarbonyl (ALOC), and the like which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, t-butyl etc. which can be removed by mild conditions compatible with the nature of the product.

The term "optional" or "optionally" means that the subsequently described event, circumstance or substituent may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

The term "ligand" as used herein denotes a compound that is a binding partner for a receptor which binding has biological significance and which is bound thereto, e.g., by complementarity. Suitable receptors include, by way of example, macromolecular

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structures, cellular receptors, cell membrane transporters, and enzymes/enzyme substrates. The specific region or regions of the ligand that is (are) recognized by the receptor is designated as the "ligand domain". A ligand may be either capable of binding to a receptor by itself, or may require the presence of one or more non-ligand components for binding (e.g., Ca^{+2} , Mg^{+2} or a water molecule is required for the binding of a ligand to various ligand binding sites).

Those skilled in the art will appreciate that portions of the ligand structure that are not essential for specific molecular recognition and binding activity may be varied substantially, replaced or substituted with unrelated structures (for example, with ancillary groups as defined below) and, in some cases, omitted entirely without affecting the binding interaction. The primary requirement for a ligand is that it has a ligand domain as defined above. It is understood that the term ligand is not intended to be limited to compounds known to be useful in binding to receptors (e.g., known drugs). Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not normally associated with receptor binding properties. In addition, it should be noted that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multibinding compounds because of the benefits conferred by multivalency.

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A "cellular receptor" is a subset of receptors and refers to cellular biological structures with one or more binding domains that reversibly complexes one or more ligands, where that complexation has biological consequences. Cellular receptors are distinguished for the purpose of this application from enzymes, which bind and then transform the bound species.

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It should be recognized that receptors that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations (e.g. cellular receptors may be covalently joined in a single structure, noncovalently associated in a multimeric structure, embedded in a membrane or

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polymeric matrix and so on) and therefore have less translational and rotational freedom than if the same cellular receptors were present as monomers in solution.

Examples of cellular receptors, ligands which bind thereto, and disease conditions mediated by these ligands include those set forth in the table below:

ligand	receptor	disease conditions treated
ipratropium (Atrovent)	muscarinic acetylcholine agonist	acute treatment of asthma
atenolol (Tenormin)	β 1 adrenergic	chronic care of coronary artery disease
losartan (Cozaar)	AT1 angiotensin	treatment of hypertension and congestive heart failure
calcitonin (calcimar)	calcitonin receptor	osteoporosis
haloperidol (Haldol)	D2 dopaminergic	care of various psychotic conditions
nafarelin (Synarel)	gonadotropic-releasing-factor receptor	treatment of endometriosis
rantidine (Zantac)	H2 histaminergic	treatment of gastric ulcers and gastroesophageal reflux disease
sumatriptan (Imitrex)	5-HT ID serotonergic	treatment of migraine disorders
zafirlukast (Acolate)	leukotriene receptor	anti-inflammatory agent in chronic treatment of asthma
morphine (kadian)	μ selective opioid receptor	increasing tolerance for pain
oxytocin (Syntocinon)	oxytocin agonist	useful in induction and maintenance of labor
misoprostol (cytotec)	prostaglandin	treating duodenal and gastric ulcers
octreotide (Sandostatin)	somatostatin	treatment of endocrinological cancers
DDAVP (desmopressin)	vasopressin	diuretic for treatment of cardiovascular disease

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The term "macromolecular structures" refers to cellular, extracellular and/or microbial components comprising 2 or more repeating structural subunits. Such

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molecules are often components of cellular construction such as components of cell envelopes (e.g., phospholipid, sterol or lipid, protein, glycolipid, and glycoprotein components), internal cytoskeletal structures (e.g., fibrils, filaments, etc.), polysome membranes (e.g., cellular and extracellular), vesicles, organelles, and matrices (intracellular and extracellular). Macromolecular structures also may include structural components of microbial particles such as viruses. In other cases, these molecules may be important in maintaining or modulating cellular function. Such structures may include proteins which contain homo- and hetero-oligomeric repeating units such as endogenous or exogenous transcription factors. On the other hand, as used herein, macromolecular structures do not include DNA, RNA, antibodies, or antibody fragments.

The macromolecular structure targeted by the ligand can be found on/in endogenous mammalian cells or on/in exogenous sources (e.g., bacterial, viral, fungal, parasitic, sources). Binding between the ligands of the multibinding compounds of this invention and the ligand binding sites on the macromolecular structure result in modulation or disruption of the biological process/function of cells and, in some cases, can lead to cell death.

Examples of macromolecular structures, ligands which bind thereto and disease conditions mediated by these ligands include those set forth in the table below:

Drug/Ligand	Macromolecular Structure Targeted by Drug	Disease Condition Treated
Amphotericin B	cell wall sterol component (principally ergosterol) of fungi	fungal infections (candidiasis, aspergillosis, cryptococcosis, etc.)
Polymyxin and colistin	bacterial cell wall	Gram negative bacterial infections (E. coli, Pseudomonas, etc.)
Pirodivir and Pleconaril	capsid protein of picornaviruses	picornaviral infections (rhinovirus, hepatitis A, echovirus meningitis, etc.)

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Drug/Ligand	Macromolecular Structure Targeted by Drug	Disease Condition Treated
Docetaxel, Paclitaxol (taxol)	tubulin and/or microtubules	Cancer (breast and ovarian carcinomas)
Griseofulvin	tubulin and/or microtubules	fungal infections
Colchicine	tubulin and/or microtubules	gout, gouty arthritis
5 Vinblastine, Vincristine, Vinorelbine, and Vindesine	tubulin and/or microtubules	Cancer (Hodgkin's disease, other lymphomas, testicular carcinomas)

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The term "enzyme" refers to enzymes, as categorized by The International Union of Biochemistry and Molecular Biology (IUBMB), including the following:

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Oxidoreductases

Transferases

Hydrolases

Lyases

Isomerases

Ligases (synthetases)

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1. Oxidoreductases catalyze reactions that involve the transfer of electrons between substrates and transition metal ions or organic cofactors. For example, sterol 14-alpha methyl demethylase, a heme-containing, membrane-associated enzyme, catalyzes the oxidative removal of a methyl group from lanosterol during the course of sterol biosynthesis in fungal and mammalian cells. The terminal acceptor of electrons in this reaction is the oxidized form of a nicotinamide-adenine dinucleotide cofactor. Specific inhibition of the fungal demethylase enzyme by azoles such as ketoconazole, itraconazole, and fluconazole forms the basis for effective antifungal therapy. A second example of an oxidoreductase enzyme is squalene monooxygenase, which is responsible

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for the conversion of squalene to (3*S*)-2,3-oxidosqualene in plant, fungal, and mammalian cells. This reaction is iron-dependent and requires dioxygen and a reduced flavin cofactor. Selective inhibition of the fungal enzyme by the allylamine family of inhibitors (including naftifine and terbinafine) is used for topical treatment of a variety of fungal infections. A third example of an oxidoreductase enzyme is cyclooxygenase (prostaglandin H2 synthase), which catalyzes the conversion of arachidonic acid to prostaglandin G2 and subsequently prostaglandin H2. There are two isoforms of this enzyme, the constitutively expressed COX-1 and COX-2, which is induced in inflammatory situations. These homodimeric, C2-symmetrical, membrane-associated enzymes are the targets of the non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin, indomethacin, ibuprofen, and naproxen. A fourth example of an oxidoreductase enzyme is dihydrofolate reductase (DHFR), which catalyzes the conversion of dihydrofolate polyglutamate to tetrahydrofolate polyglutamate using a reduced nicotinamide cofactor. DHFR is the primary site of action of antifolate cancer chemotherapeutics (e.g., methotrexate) and parasite chemotherapeutics (trimetrexate). A fifth example of an oxidoreductase is the homodimeric integral membrane enzyme hydroxymethyl glutaryl-coenzyme A reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-determining step in sterol biosynthesis. Inhibition of this enzyme by the statins (e.g., lovastatin, atorvastatin) leads to reduction of serum cholesterol and triglyceride levels.

2. Transferase enzymes catalyze, among other things, the primary processes by which biological macromolecules are biosynthesized. DNA and RNA are formed through sequential phosphoryl group transfers, proteins are formed through sequential acyl group transfers, and polysaccharides are formed through sequential glycosyl group transfers. These processes constitute primary targets of a variety of anticancer and antimicrobial agents. The process catalyzed by retroviral reverse transcriptase is inhibited by enzyme-mediated incorporation of activated forms of nucleotide analogs such as azidothymidine (AZT) by the procedure known as substrate adulteration. In a second example, DNA-dependent RNA polymerase in mycobacterial cells is selectively inhibited by the antibacterial agent rifampin. In a third example, protein biosynthesis in

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bacterial cells is selectively inhibited by a variety of antibiotics (including gentamicin and other aminoglycosides, tetracycline, chloramphenicol, erythromycin and other macrolides, clindamycin) that target binding sites on the ribosome, a multicomponent ribonucleoprotein complex that catalyzes the energy- and mRNA-dependent transfer of amino acid subunits on aminoacyl tRNAs to growing polypeptide chains. In a fourth example, vancomycin and other glycopeptide antibiotics inhibit the multifunctional, membrane-bound transglycosylase responsible for the glycosyl transferase reaction which incorporates lipid intermediate II into the growing bacterial cell wall. Vancomycin acts via a substrate sequestration mechanism; i.e., it binds to and sequesters lipid intermediate II within a complex that is not recognized by the transglycosylase. In a fifth example, the large family of beta-lactam antibiotics inhibit the action of a series of penicillin binding proteins (PBPs), most notably multifunctional, membrane-bound transpeptidase enzymes that are responsible for cross-linking of the bacterial cell wall.

3. Hydrolase enzymes mediate particular subsets of transfer reactions in which moieties are transferred to a water molecule partner. These reactions are central to the metabolism of biological macromolecules including nucleic acids, proteins, and polysaccharides. Hydrolysis reactions are also important for metabolism of smaller molecules. For example membrane-bound undecaprenyldiphosphatase catalyzes the hydrolysis of undecaprenyl pyrophosphate to undecaprenyl phosphate, which is then available for reuse as the lipid carrier for intermediates involved in bacterial cell wall biosynthesis. The topical antibacterial agent bacitracin inhibits the diphosphatase process by sequestering the substrate, analogous to the mode of action of vancomycin. In a second example, beta-lactamases are responsible for ring-opening hydrolytic inactivation of beta-lactam antibiotics. These soluble, monomeric enzymes are irreversibly acylated by clavulanic acid and sulbactam, which can be used as an adjunct to beta-lactam antibiotics in antibacterial therapy. A third example of a hydrolase enzyme is the aspartyl protease from HIV, which is a homodimer of 99 amino acid subunits which combine to form a single active site. A fourth example of a hydrolase enzyme is the homotetrameric viral neuraminidase, which catalyzes the hydrolytic removal of terminal sialic acid groups from polysaccharides on mammalian cells which serve as adhesion ligands for the

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virus. Inhibition of viral neuraminidase by agents such as GS4104 represents a promising approach to the prophylaxis and therapy of infections due to influenza virus.

4. Lyase enzymes catalyze the addition of groups to double bonds, or the removal of groups to form double bonds. Examples include carbonic anhydrase, aspartase (which catalyzes the removal of ammonia from aspartic acid to form fumaric acid), and 5-dehydroquinate dehydrase (which catalyzes the dehydrative conversion of 5-dehydroquinate to 5-dehydroshikimate).

10 5. Isomerases catalyze rearrangements in the covalent framework of a substrate without changing its formal composition. One example of an isomerase enzyme is the homodimeric, membrane-associated lanosterol synthase, which catalyzes the complex cyclization/rearrangement of oxidosqualene to the first carbocyclic intermediate in mammalian and fungal sterol biosynthesis pathways. A second example
15 of an isomerase is thromboxane synthase, which catalyzes the conversion of prostaglandin H₂ to thromboxane A₂. This process is inhibited by dazoxiben and piroxicam. A third example of an isomerase is alanine racemase, a soluble, monomeric bacterial enzyme that produces D-alanine for incorporation into the cell wall. A fourth example of an isomerase is the family of topoisomerase enzymes, which are responsible
20 for relaxing superhelical stress in double-stranded DNA molecules as they undergo transcription and replication. These enzymes are important targets of antimicrobial agents, in particular the quinolones, which inhibit the $\alpha_2\beta_2$ tetrameric topoisomerase II (DNA gyrase) in bacterial cells, and also etoposide, and teniposide, which inhibit the mammalian topoisomerase II enzyme.

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6. Ligase enzymes join two molecules concomitant with the cleavage of a phosphoric anhydride linkage, most usually that of ATP. One example of a ligase enzyme is D-alanyl-D-alanine ligase, which mediates the ATP-dependent condensation of two D-alanine subunits as an early step in bacterial cell wall biosynthesis.

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Nearly all known enzymes are proteins. As is true for all proteins, the structures of enzymes may be described at several different levels. The primary structure consists

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of an unbranched polypeptide chain derived from head-to-tail condensation of L-alpha-amino acids (in some instances other amino acids are incorporated). As such, enzymes have distinct amino termini and carboxyl termini. Hydrogen bonding, hydrophobic, and other interactions between and among neighboring amino acid residues typically occur within preferred conformations for the main chain atoms, affording secondary structural elements such as beta sheets and alpha helices. Specific clustering of secondary structural elements may be recognizable as supersecondary structures and/or as part of independently folded domain units of an enzyme, leading eventually to the tertiary structure, the "arrangement in space of all atoms in a single polypeptide chain or in covalently linked chains." (Fersht)

Non-covalent oligomerization of protein subunits in enzymes is common, and the organization of these subunits is referred to as the quaternary structure. A great variety of enzyme quaternary structures are possible through variation in the numbers and identities of subunits. The simplest enzyme quaternary structures are those wherein two identical polypeptide chains combine to form an enzyme with a single active site. An example of such a structure is the homodimeric HIV protease. Lanosterol synthase, prostaglandin H2 synthase, and angiotensin converting enzyme are examples of homodimeric enzymes with two distinct ligand recognition sites. The simplest heteromeric structure is a heterodimer, exemplified by the alpha-beta structure of farnesyl-protein transferase, which is responsible for farnesylation of Ras and other proteins. A higher-order structure is exemplified by alpha2-beta2 heterotetrameric DNA topoisomerase II, whose quaternary structure and two active sites is logical with respect to the pseudo-C2 symmetry of its DNA substrate and the nicking/passing/closing reactions catalyzed by this enzyme. At the far end of quaternary structural complexity is the eukaryotic ribosome, which contains four distinct rRNA strands and up to 100 individual polypeptides.

Enzymes (and enzymatic processes) are multivalent in nature when they bear two or more ligand recognition sites associated through the covalent structure of the enzyme or through the formation of a quaternary structure. They may also be multivalent by

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virtue of having at least one active site and at least one distinct non-substrate ligand-binding site associated through the covalent structure of the enzyme or through the formation of a quaternary structure, are associated with a common surface (e.g., a cell membrane), and/or utilize multivalent substrates (e.g., substrates bearing multiple transformable groups and/or substrates associated with a common surface). Although not wishing to be bound or restricted by any particular theory or proposed mechanism of action, it is believed that as a consequence of the interrelated structure of enzymes, the multibinding agents of the invention can be made by utilizing any ligand that is recognized by a multivalent enzyme or enzyme substrate.

Examples of enzymes and enzymatic processes mediated by ligands (drugs) which bind thereto and disease conditions mediated by these drugs include those set forth in the table below:

Enzyme/ Enzymatic Process	Structural Organization	Therapeutic Indication(s)	Inhibitory Drug(s)
Oxidoreductase Enzymes			
HMG-CoA reductase (1.1.1.34)	Membrane-bound homodimer	Hypercholesterolemia	Mevastatin, lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin
3-Beta-hydroxysteroid dehydrogenase (1.1.1.51)		Anti-adrenocorticosuric	Trilostane
15-hydroxyprostaglandin dehydrogenase (1.1.1.141)	Dimer		
Inosine 5'-phosphate dehydrogenase (1.1.1.245)	Homohexamer/ Homotetramer	Anti-epitaxial syncytial virus	Ribavirin (monophosphate form)
Glycerol phosphate oxidase (1.1.3.21)		Anti-protozoal	Suramin
Succinic semialdehyde dehydrogenase (1.2.1.16)	Multimer	Complex partial seizures	Valproic acid
Steroid 5-alpha reductase 2 (1.3.99.5)		Prostatic hypertrophy Male-pattern baldness	Finasteride
Steroid 5-alpha reductase 1 (1.3.99.5)			
Monamine oxidase (1.4.3.6)	Membrane-bound dimer	Migraine Depression	Phenelzine, isocarboxazid, nialamylpyromine, selegiline
Dihydrofolate reductase (microbial) (1.5.1.3)		Anti-parasite Anti-bacterial	Chloroguanide, pyrimethamine Trimethoprim
Dihydrofolate reductase (1.5.1.3)	Monomer	Anti-cancer Psoriasis	Methotrexate
Trypanothione reductase (1.6.4.8)		Anti-protozoan	Melarsoprol
Peroxidase (1.11.1.7)		Hyperthyroidism	Propylthiouracil, methimazole, carbimazole
12-Lipoxygenase (1.13.11.31)	Membrane-bound?		
5-Lipoxygenase (1.13.11.34)	Membrane-bound?	Inflammation	Zileuton, docosanone, ICI-D2318, MK-0591, MK-886 piripost, tinidap
Nitric Oxide Synthase (1.14.13.39)	Homodimer		
Cyclooxygenase 1 (1.14.99.1)	Membrane-associated homodimer	Inflammation	NSAIDs, aspirin, ibuprofen, flurbiprofen, indomethacin, acetaminophen, tolnmetin, mefenamic acid
Cyclooxygenase 2 (1.14.99.1)	Membrane-associated homodimer (inflamed tissue)	Inflammation	See above
Squalene monooxygenase (1.14.99.7)	Membrane-bound	Anti-fungal	Terbinafine
Cytochrome P450-dependent sterol 14-alpha methyl demethylase (Cytochrome P450 17-alpha or Cytochrome P450 scs enzyme) (1.14.99.9 or 1.14.15.6)	Membrane-bound complex with flavoprotein	Anti-fungal Anti-parasitical	Ketoconazole, fluconazole, itraconazole, clotrimazole, miconazole
Cytochrome P450 11-beta		Anti-cancer (hypercorticism in adrenal neoplasm or ectopic ACTH production)	Metyrapone (METPIRONE)
Ribonucleoside diphosphate	Heterodimer	Anti-cancer	Hydroxyurea

reductase (1.17.4.1) Aromatase		(myeloproliferative disorders) Cushing's syndrome Metastatic breast cancer	Aminoglutethimide (Cytadren)
Transferase Enzymes			
Catechol-O-methyl transferase (2.1.1.6)			
Glycylamide ribonucleotide Transferase (2.1.2.2)		Anti-cancer (Acute lymphocytic leukemia, choriocarcinoma, mycosis fungoides, breast, head and neck, osteogenic sarcoma)	5,8-Dideazatetrahydrofolic acid (DDATHF), methotrexate
Ribosomal protein biosynthesis (50S ribosomal subunit) (2.3.2.12)	Multisubunit ribonucleoprotein particle	Anti-bacterial	Chloramphenicol Erythromycin, clarithromycin, azithromycin, dithromycin Clindamycin, lincomycin Streptogramins (Synercid)
Ribosomal protein biosynthesis (Aminoacyl tRNA site on 30S ribosomal unit) (2.3.2.12)	Multisubunit ribonucleoprotein particle	Anti-bacterial	Tetracycline, chlortetracycline, oxytetracycline, demeclocycline, methacycline, doxycycline, minocycline
Ribosomal protein biosynthesis (30S subunit) (2.3.2.12)	Multisubunit ribonucleoprotein particle	Anti-bacterial	Streptomycin, gentamicin, tobramycin, amikacin, netilmicin, kanamycin, neomycin, spectinomycin
Ribosomal protein biosynthesis (soluble protein factors) (2.3.2.12)	Multisubunit ribonucleoprotein particle	Anti-bacterial	Fusidic acid
Hypoxanthine-guanine phosphoribosyltransferase (2.4.2.9)			Purine analogs
Dihydropteroate synthase (2.5.1.15)		Anti-bacterial	Sulfanilamide, sulfamethoxazole, sulfacetamide, sulfadiazine, sulfisoxazole, sulfasalazine, sulfadoxine
Leukotriene C-4 synthase (2.5.1.37)	Membrane-bound homodimer		Vigabatrin, valproic acid
GABA transaminase (2.6.1.19)			
Guanylate kinase (2.7.4.8)	Heterodimer		
Beta subunit of DNA-dependant RNA polymerase (2.7.7.6)	Heteromultimer		Rifampin
Reverse transcriptase (2.7.7.49)	Heterodimer		Zidovudine, didanosine, zalcitabine, zalcitabine
D,D-transpeptidases (PBP 2a, 2b)	Membrane-bound bifunctional transglycosylase/transpeptidase enzyme, may be part of multimeric structure		Beta-lactams (Penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, carbenicillin, carbenicillin indanyl, ticarcillin, mezlocillin, piperacillin, cephalothin, cefazolin, cephalexin, cefadroxil, cefamandole, cefazolin, cefaclor, cefuroxime, loracarbef, cefonicid, cefotetan, ceforanide, cefotaxime, cefpodoxime proxetil, ceftioxcime, ceftioxcime, cefoperazone, ceftazidime, cefepime, imipenem, meropenem, aztreonam)

D,D-transpeptidases (PBP 1a)	Membrane-bound monomer		Beta-lactams (see above)
Transglycosylase (2.4.1.129)	Membrane-bound bifunctional transglycosylase/transpeptidase enzyme, may be part of multimeric structure		Vancomycin, teicoplanin (Vancomycin's target is not the poorly characterized diphosphatase but rather the membrane-bound undecaprenyldiphosphate substrate)
Hydrolase Enzymes			
Phospholipase A2 (3.1.1.4)	Membrane-bound/calcium- dependent		
Acetylcholinesterase (3.1.1.7)	Homooligomers with multiple binding sites per monomer unit	Glaucoma Gastrointestinal and bladder motility Myasthenia gravis Alzheimer's disease	(-) Huperzine A, edrophonium, neostigmine, propidium, fasciculin, neostigmine, physostigmine, demecarium, ambenonium, diisopropyl fluorophosphate (DFP), echothiophate
Cholinesterase (3.1.1.8)	Homologous to acetylcholinesterase	Schistosomiasis	metrifonate
Phospholipase C (3.1.4.3)	Membrane-bound monomer		
Type III cyclic AMP phosphodiesterase (3.1.4.17)	Probable multimer	Heart failure	Milrinone, amrinone, pirobendan, cilostamide, enoximone, peroximone, vesnarinone
Type V cyclic nucleotide phosphodiesterase (3.1.4.17)	Probable multimer	Anti-platelet aggregation	Zaprinast, dipyridamole
Type I cyclic nucleotide phosphodiesterase (3.1.4.17)	Homodimer	CNS modulation vasorelaxation	Vinpocetine
Type IV Cyclic AMP Phosphodiesterase (3.1.4.17)	Soluble Homodimer	Airway smooth muscle relaxation Inhibition of inflammatory intermediary release CNS modulation Gastric acid secretion Starch absorption	Rolipram, RO-020-1724
Glucoamylase (3.2.1.3)			Acarbose
Neuraminidase (3.2.1.16)	Homotetramer, either membrane bound or bound on surface of viral particle		Zanamivir, GS4104
Alpha-glucosidase (3.2.1.20)		Starch absorption	Acarbose
Sucrase (3.2.1.48)		Starch absorption	Acarbose
Leukotriene A hydrolase (3.3.2.6)			
Angiotensin-converting enzyme (3.4.13.1)	Homodimer	Hypertension Left ventricular systolic dysfunction Myocardial infarction Scleroderma renal crisis	Captopril, lisinopril, pralopril, zofenopril, alacepril Enalapril, lisinopril, benazepril, quinapril, moexipril, ramipril, spirapril, perindopril, indolapril, pentopril, indalopril, cilazapril
D,D-Carboxypeptidase (PBP 3-7) (3.4.16.4)	May be part of multisubunit structures	Anti-bacterial	fosinopril Beta-lactams (see above)
Coagulation Proteases Factor IXa (3.4.21.22) Factor VIIa (3.4.21.21)	Membrane-associated during activation/function. Activation occurs through multi-subunit complexes on cell surfaces	Anti-coagulation	Heparin; low molecular weight heparin

Factor Xa (3.4.21.6) Thrombin (3.4.21.9)			
Hemoglobin protease (Plasminogen I and II EC 3.4.23.38, 3.4.23.39)		Anti-malarial	Chloroquine, quinine mefloquine, halofantrine
Neutral endopeptidase (3.4.24.11)		Volume-expanded hypertension	Dual ACE/NEP inhibitors in development
Beta-Lactamase (3.5.2.6)	Several classes of typically soluble, monomeric enzymes. In Gram- negative organisms, they are confined to the periplasmic space	Anti-bacterial adjunct therapy	Cloxacillin, sulbactam, tazobactam
Adenosine deaminase (3.5.4.4)		Anti-cancer (Hairy cell leukemia, mycosis fungoides, chronic lymphocytic leukemia)	Pentostatin; erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA)
Undecaprenyldiphosphatase (3.6.1.27)	Membrane-bound substrate as target	Anti-bacterial	Bactracin (Target is not the poorly characterized diphosphatase but rather the membrane-bound undecaprenyldiphosphate substrate)
Iodothyronine-5'-deiodinase, type I (3.8.1.4)	Multimeric protein	Hyperthyroidism	Propylthiouracil, methimazole, carbimazole, amiodarone
Iodothyronine-5'-deiodinase, type II (3.8.1.4)	Multimeric protein	Hyperthyroidism	Propylthiouracil, methimazole, carbimazole, amiodarone
Lyase Enzymes			
Glutamic acid decarboxylase (4.1.1.15)		Epileptic seizure mania	Valproic acid (actually stimulates the enzyme)
Type II carbonic anhydrase (4.2.1.1)	Soluble monomer	Glaucoma Edema associated with congestive heart failure	Acetazolamide, dichlorophenamide, napatane
Type IV carbonic anhydrase (4.2.1.1)	Membrane bound (may be dimer)	Diuretics	Acetazolamide, methazolamide, dichlorophenamide
Isomerase Enzymes			
L-Alanyl racemase (5.1.1.1)	Monomer or dimer, depending upon bacterial strain	Anti-bacterial	D-Cycloserine
Prostacyclin synthase (5.3.99.4)	Soluble monomer		
Thromboxane synthase (5.3.99.5)	Membrane-bound monomer	Anti-platelet aggregation Anti-vasoconstriction (clinical efficacy in question)	Dazoxiben, piroxicam, nifedipine
Microbial oxidosqualene- lanosterol cyclase enzymes (5.4.99.7)	Membrane-bound homodimer	Potential for: Anti-fungal Anti-parasite Anti-cholesterol	Research and pre-clinical
DNA gyrase subunit alpha/beta (5.99.1.3)	Alpha2Beta2 heterotetramer	Anti-bacterial	Nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin, cinoxacin, sparfloxacin, lomefloxacin, fleroxacin, pefloxacin, amifloxacin, novobiocin
Topoisomerase II (5.99.1.3)	Homodimer (unlike bacterial enzyme?)	Anti-cancer (testicular, lung, breast, Hodgkin's disease, non-Hodgkin's lymphoma, acute granulocytic leukemia, Kaposi's sarcoma)	Etoposide, teniposide
Fungal isomerase enzymes	Membrane-bound?	Anti-fungal (dermatophytes)	Tolnaftate
Ligase Enzymes			
D-Alanyl-D-alanine ligase (6.3.2.4)	Homodimer		

<i>Unclassified Enzymes</i>			
Plasmodial home polymerase		Anti-malarial	Chloroquine, quinine mefloquine, halofantrine

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The term "cell membrane transporter" refers to a membrane-associated transporter of ions and/or molecules to which the lipid membrane is normally impermeant. Transporters include ion channels, molecular transporters and ion pumps. Examples of cell membrane transporters, ligands (drugs) that bind thereto and disease conditions mediated thereby include those set forth below:

Channel	Clinical Indication	Drug	Binding Site	References
Shaker K ⁺	Arrhythmia	Amiodarone Quinidine	AA residues on S6 segment of K ⁺ channel	Grace et al. NEJM 338(1); 35 (1998)
Na ⁺	Local Anesthesia	Lidocaine Bupivacaine Ropivacaine Procaine Chloroprocaine Etidocaine Mepivacaine Benzocaine	AA residues on IV/S6 of Na ⁺ channel	Ragsdale et al. PNAS 93; 9270 (1996)
	Arrhythmia	Flecainide Mexiletine Morcizine Tocainide	AA residues on IV/S6 of Na ⁺ Channel	Ragsdale et al. PNAS 93; 9270 (1996)
	Epilepsy	Phenytoin	AA residues IV/S6 of Na ⁺ Channel	Ragsdale et al. PNAS 93; 9270 (1996)
	Hypertension	Amlodipine Felodipine Isradipine Nicardipine Nifedipine Nimodipine Nisoldipine Verapamil	DHP binding site – amino acids on IIIS5/IIIS6 and IVS6 of Ca ²⁺ channel	Striessnig et al. TIPS 19; 108 (1998)
Ca ²⁺	Hypertension; Heart Failure		PAA binding site – amino acid residues on IVS6 of Ca ²⁺ channel	Striessnig et al. TIPS 19; 108 (1998)
	Arrhythmia	Bepredil		
5HT ₃	Emesis	Ondansetron Granisetron Dolasetron		
GABA	Epilepsy	Benzodiazepines Alprazolam Brotizolam Chlordiazepoxide Clobazam Clonazepam Clorazepate Demoxepam Diazepam Estazolam Flumazenil Flurazepam Halazepam Lorazepam Midazolam Nitrazepam	BDZ binding site on the α subunit	Sigel et al. JBC 258; 6965; (1983)

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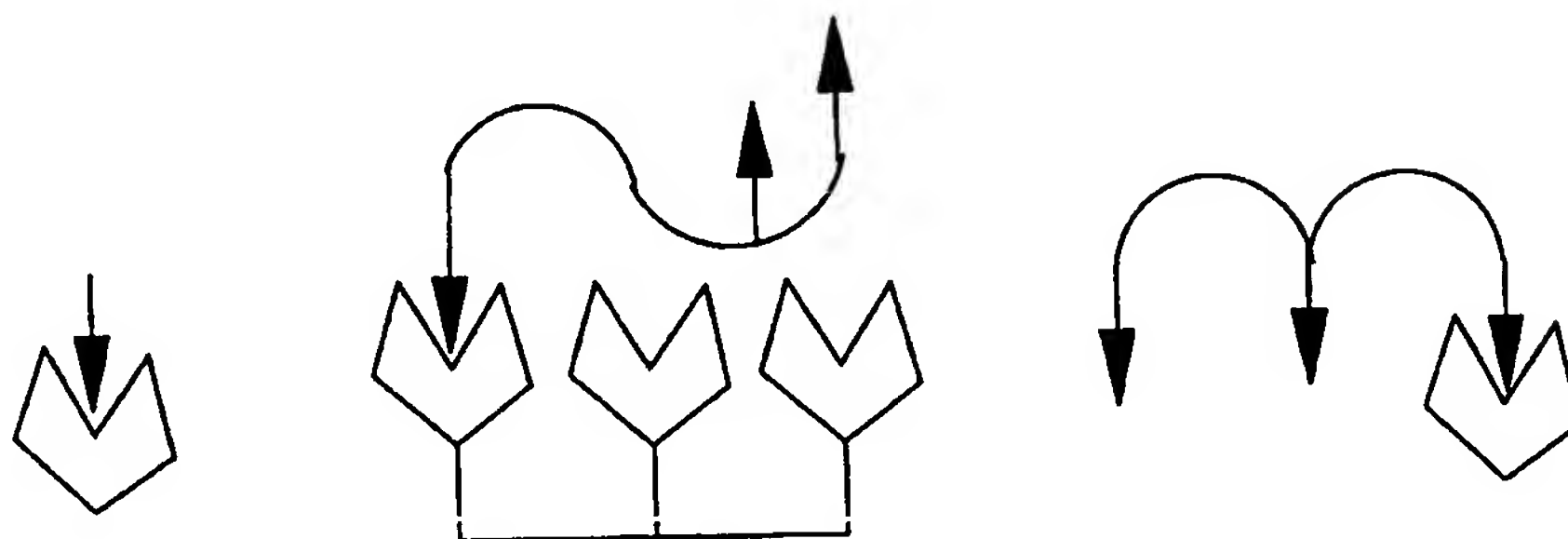
The term "multibinding compound or agent" refers to a compound that is capable of multivalency, as defined below, and which has 2-10 ligands covalently bound to one or more linkers which may be the same or different. Multibinding compounds provide a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which are made available for binding. That is to say that the biological and/or therapeutic effect of the ligands attached to the multibinding compound is greater than that achieved by the same amount of unlinked ligands made available for binding to the ligand binding sites (receptors). The phrase "increased biological or therapeutic effect" includes, for example: increased affinity, increased selectivity for target, increased specificity for target, increased potency, increased efficacy, decreased toxicity, improved duration of activity or action, decreased side effects, increased therapeutic index, improved bioavailability, improved pharmacokinetics, improved activity spectrum, and the like. The multibinding compounds of this invention will exhibit at least one and preferably more than one of the above-mentioned affects and, accordingly, a multimeric compound possesses multibinding properties when it exhibits one or more of these affects.

The term "potency" refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multibinding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g., in an *in vitro* or *in vivo* assay, in an appropriate animal model). The finding that the multibinding agent produces an equivalent biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand is indicative of enhanced potency.

The term "univalency" as used herein refers to a single binding interaction between one ligand as defined herein with one ligand binding site as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibits

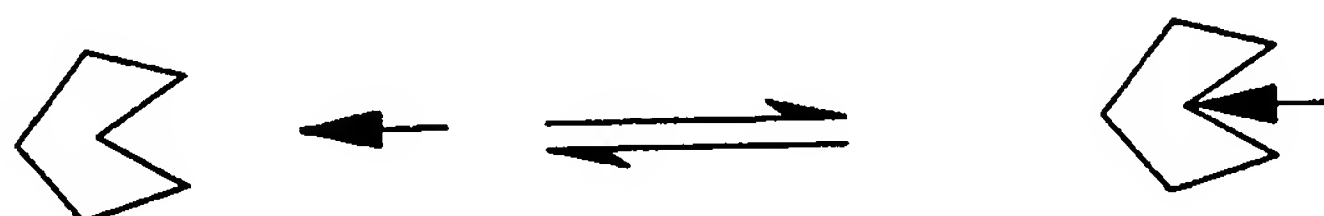
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univalency when only one ligand is interacting with a ligand binding site. Examples of univalent interactions are depicted below.

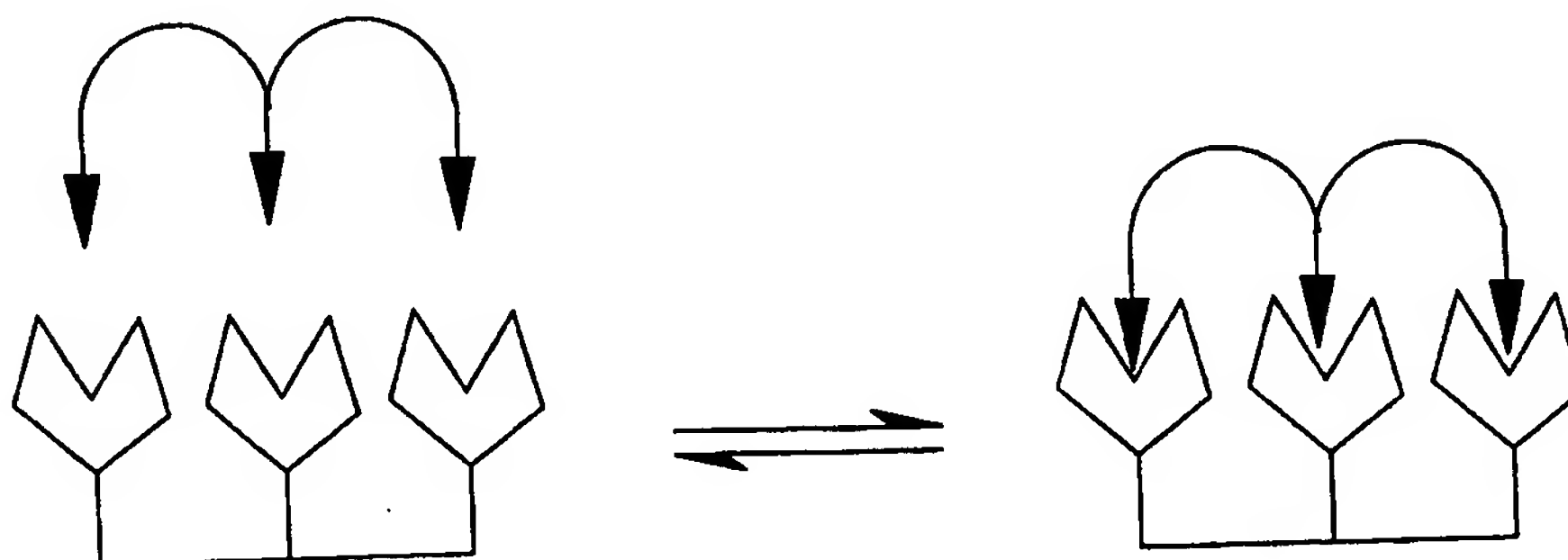


The term "multivalency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands (which may be the same or different) and two or more corresponding receptors (ligand binding sites) which may be the same or different.

For example, two ligands connected through a linker that bind concurrently to two ligand binding sites would be considered as bivalency; three ligands thus connected would be an example of trivalency. An example of trivalent binding, illustrating a multibinding compound bearing three ligands versus a monovalent binding interaction, is shown below:



Univalent Interaction



Trivalent Interaction

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It should be understood that not all compounds that contain multiple copies of a ligand attached to a linker or to linkers necessarily exhibit the phenomena of multivalency, i.e., that the biological and/or therapeutic effect of the multibinding agent is greater than the sum of the aggregate of unlinked ligands made available for binding to the ligand binding site (receptor). For multivalency to occur, the ligands that are connected by a linker or linkers have to be presented to their ligand binding sites by the linker(s) in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multibinding event.

The term "selectivity" or "specificity" is a measure of the binding preferences of a ligand for different ligand binding sites (receptors). The selectivity of a ligand with respect to its target ligand binding site relative to another ligand binding site is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex) or, in cases where a biological effect is observed below the K_d , the ratio of the respective EC_{50} 's (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct ligand binding sites (receptors)).

The term "ligand binding site" denotes the site on the receptor that recognizes a ligand domain and provides a binding partner for the ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example, agonism, antagonism, modulatory effects, may maintain an ongoing biological event, and the like.

The terms "agonism" and "antagonism" are well known in the art. The term "modulatory effect" refers to the ability of the ligand to change the activity of an agonist or antagonist through binding to a ligand binding site.

It should be recognized that the ligand binding sites that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and inter-molecular associations (e.g., such structures may be covalently joined to a single structure, noncovalently associated in a multimeric structure, embedded in a membrane or

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polymeric matrix, and so on) and therefore have less translational and rotational freedom than if the same structures were present as monomers in solution.

The term "treatment" refers to any treatment of a pathologic condition in a mammal, particularly a human, and includes:

- (i) preventing the pathologic condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the disease condition;
- (ii) inhibiting the pathologic condition, i.e., arresting its development;
- (iii) relieving the pathologic condition, i.e., causing regression of the pathologic condition; or
- (iv) relieving the conditions mediated by the pathologic condition.

The term "pathologic condition which is modulated by treatment with a ligand" covers all disease states (i.e., pathologic conditions) which are generally acknowledged in the art to be usefully treated with a ligand for the receptor in general, and those disease states which have been found to be usefully treated by a specific multibinding compound of our invention. Such disease states include, by way of example only, the treatment of a mammal afflicted with disease conditions recited above.

The term "therapeutically effective amount" refers to that amount of multibinding compound which is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "substrate" or "solid support" refers to a material having a rigid or semi-rigid surface which contains or can be derivatized to contain reactive functionality which covalently links a compound to the surface thereof. Such materials are well known in the art and include, by way of example, silicon dioxide supports containing reactive Si-OH

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groups, polyacrylamide supports, polystyrene supports, polyethyleneglycol supports, and the like. Such supports will preferably take the form of small beads, pellets, disks, or other conventional forms, although other forms may be used. In some embodiments, at least one surface of the substrate will be substantially flat.

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In one embodiment, the ligand or linker is covalently attached directly to the solid support or is attached to the support via a spacer arm which spaces the ligand or linker away from the solid support. Spacing arms are well known in the art and include, by way of example only, conventional spacing arms such as those comprising ester, amide, carbamate, ether, thio ether, urea, amine groups and the like. The spacing arm can also be a covalent bond. The spacing arm can be cleavable or non-cleavable.

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"Cleavable spacing arms" refer to spacing arms wherein at least one of the covalent bonds of the spacing arm which attaches the compound to the solid support can be readily broken by specific chemical reactions thereby providing for multimeric compounds comprising at least 2 ligands linked via at least 1 linker free of the solid support ("soluble compounds"). The chemical reactions employed to break the covalent bond of the spacing arm are selected so as to be specific for bond breakage thereby preventing unintended reactions occurring elsewhere on the compound. The cleavable spacing arm is selected relative to the synthesis of the compounds to be formed on the solid support so as to prevent premature cleavage of this compound from the solid support as well as not to interfere with any of the procedures employed during compound synthesis on the support. Suitable cleavable spacing arms are well known in the art.

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"Non-cleavable spacing arms" refer to spacing arms wherein the covalent bond(s) spacing the ligand or linker to the solid support can only be cleaved under conditions which chemically alters unintended parts of the structure of the compound attached thereto.

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The term "substantially homogeneous" refers to collections of molecules wherein at least 80%, preferably at least about 90% and more preferably at least about 95% of the molecules are a single compound or stereoisomers thereof.

5 The term "stereoisomer" refers to a chemical compound having the same molecular weight, chemical composition, and constitution as another, but with the atoms are arranged differently in space. That is, certain identical chemical moieties are at different orientations in space and, therefore, when pure, have the ability to rotate the plane of polarized light. However, some pure stereoisomers may have an optical rotation
10 that is so slight that it is undetectable with present instrumentation. The compounds described herein may have one or more asymmetrical carbon atoms and therefore include various stereoisomers or may be diastereo isomers such as *cis* and *trans*. All stereoisomers are included within the scope of the invention.

15 When chiral centers are found in the multimers of this invention, it is to be understood that this invention encompasses all possible stereoisomers.

 The term "removable protecting group" or "protecting group" refers to any group which when bound to a functionality such as hydroxyl, amino, or carboxyl groups
20 prevents reactions from occurring at these functional groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the functional group. The particular removable protecting group employed is not critical.

 The term "pharmaceutically acceptable salt" refers to pharmaceutically acceptable
25 salts of a compound of formula I which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, tartrate, mesylate, acetate, maleate, oxalate and the
30 like.

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The term "linker", identified where appropriate by the symbol X, refers to a group or groups that covalently links from 2 to 10 ligands (as identified above) in a manner that provides for a compound capable of multivalency. Among other features, the linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. In some cases, the linker may itself be biologically active. The term "linker" does not, however, extend to cover solid inert supports such as beads, glass particles, fibers, and the like. But it is understood that the multibinding compounds of this invention can be attached to a solid support if desired. For example, such attachment to solid supports can be made for use in synthesis, separation and purification processes and similar applications.

The extent to which multivalent binding is realized depends upon the efficiency with which the linker or linkers that joins the ligands presents these ligands to the array of available ligand binding sites. Beyond presenting these ligands for multivalent interactions with ligand binding sites, the linker or linkers spatially constrains these interactions to occur within dimensions defined by the linker or linkers. Thus, the structural features of the linker (valency, geometry, orientation, size, flexibility, chemical composition, etc.) are features of multibinding agents that play an important role in determining their activities.

The linkers used in this invention are selected to allow multivalent binding of ligands to the ligand binding sites, whether such sites are located interiorly, both interiorly and on the periphery of the receptor structure, or at any intermediate position thereof.

The ligands are covalently attached to the linker or linkers using conventional chemical techniques providing for covalent linkage of the ligand to the linker or linkers. Reaction chemistries resulting in such linkages are well known in the art and involve the use of complementary functional groups on the linker and ligand. Preferably, the complementary functional groups on the linker are selected relative to the functional groups available on the ligand for bonding or which can be introduced onto the ligand for

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bonding. Again, such complementary functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable, well-known activating agents results in formation of an amide bond covalently linking the
5 ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker.

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The linker is attached to the ligand at a position that retains ligand domain-ligand binding site interaction and specifically which permits the ligand domain of the ligand to orient itself to bind to the ligand binding site. Such positions and synthetic protocols for linkage are well known in the art. The term linker embraces everything that is not
15 considered to be part of the ligand.

At present, it is preferred that the multibinding agent is a bivalent compound, e.g., two ligands which are covalently linked to linker X. Such bivalent compounds are preferably represented by the formula L-X-L where each L is independently a ligand and
20 X is a bivalent linker. Suitable bivalent linkers included, by way of example only, alkylene, substituted alkylene, polyoxyalkylenes (e.g., -O-(alkylene-O-)_p, where *p* is an integer from 1 to 50) alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkylene, substituted cycloalkylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, and the like or combinations of
25 such groups, e.g., -alkylene-arylene-; alkylene-arylene-alkylene-; alkylene-cycloalkylene-; alkylene-cycloalkylene-alkylene-; -alkylene-heterocyclene-, and the like. Suitable linkers are discussed more fully below.

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General Synthetic Procedures

Methods for Preparing Libraries

In one aspect, the methods of this invention provide for a library of multimeric compounds each member of which may or may not possess multibinding properties.

- 5 Such libraries are produced by synthesizing a plurality of multimeric compounds either using solution phase or solid phase chemistry where either the ligand or the linker is attached to a solid support via a cleavable or non-cleavable spacing arm. Preferably, this spacing arm is distinct from the linker and merely serves to provide a point of attachment for the synthesized multimeric compound to the solid support. However, it is
- 10 contemplated that when spacing arm contains a multiplicity of reactive functionalities, the spacing arm can also serve as the linker.

- The libraries provided by this invention are synthesized by conventional methods well known in the art including use of split/pool techniques or chips having a specific
- 15 compound located at a specific site.^{9,10} The synthetic protocols for library generation employ functionalized ligands and linkers preferably taking into account relevant factors for their rational selection although random selection can also be employed.

- Specifically, such relevant factors taken into consideration include the proper
- 20 juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets. This is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters
- 25 that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the identity of the linker, (4) the valency of the linker, (5) linker length, (6) linker geometry, (7) linker physical properties, and (8) linker chemical functional groups.

- 30 Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables

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are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

5 Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s) or receptor(s). Thus, ligands
10 may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients,
15 appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently
20 potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives
25 as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

30

Orientation: selection of ligand attachment points and linking chemistry

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The orientation of the ligand relative to the linker in a multimeric compound can be achieved by selection of the point at which the ligand attaches to the linker and/or the orientation and stereochemistry of the linker (i.e., racemic and isolated stereoisomers). Each of these will be discussed in turn.

5

Point of Attachment

Several points are preferably chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

25

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target

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molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or are elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the formal antagonist binding site.

It should be further understood that multibinding compounds may exhibit intrinsically new and desirable activities relative to their component ligands. For example, appropriate linking of receptor agonists and/or antagonists may provide compounds which display partial agonist properties. It should also be understood that the overall potency and degree of efficacy may be controlled and selected through choice of agonist ligands, antagonist ligands and linkers. By way of example, the joining of one or more β_2 adrenergic receptor agonists with one or more β_2 adrenergic receptor antagonists provides a compound which is a potent agonist of β_2 adrenergic receptors but with sub-maximal efficacy (i.e., it is a partial agonist). In this instance, a partial agonist is preferred in order to avoid cardiovascular side effects and tachyphylaxis.

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets. For example, a 5HT₄ receptor antagonist and a bladder-selective muscarinic M₃ antagonist may be joined to a linker through attachment points which do not abrogate the binding affinity of the monomeric ligands for their respective receptor sites. The dimeric compound may achieve enhanced affinity for both receptors due to favorable interactions between the 5HT₄ ligand and elements of the M₃ receptor proximal to the formal M₃

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antagonist binding site and between the M_3 ligand and elements of the $5HT_4$ receptor proximal to the formal $5HT_4$ antagonist binding site. Thus, the dimeric compound may be a more potent and selective antagonist of overactive bladder and a superior therapy for urinary urge incontinence. It should be recognized that the multibinding compounds of this invention may possess activities not expressed by the monomeric versions of the ligand such as agonist, antagonist or partial agonist behavior.

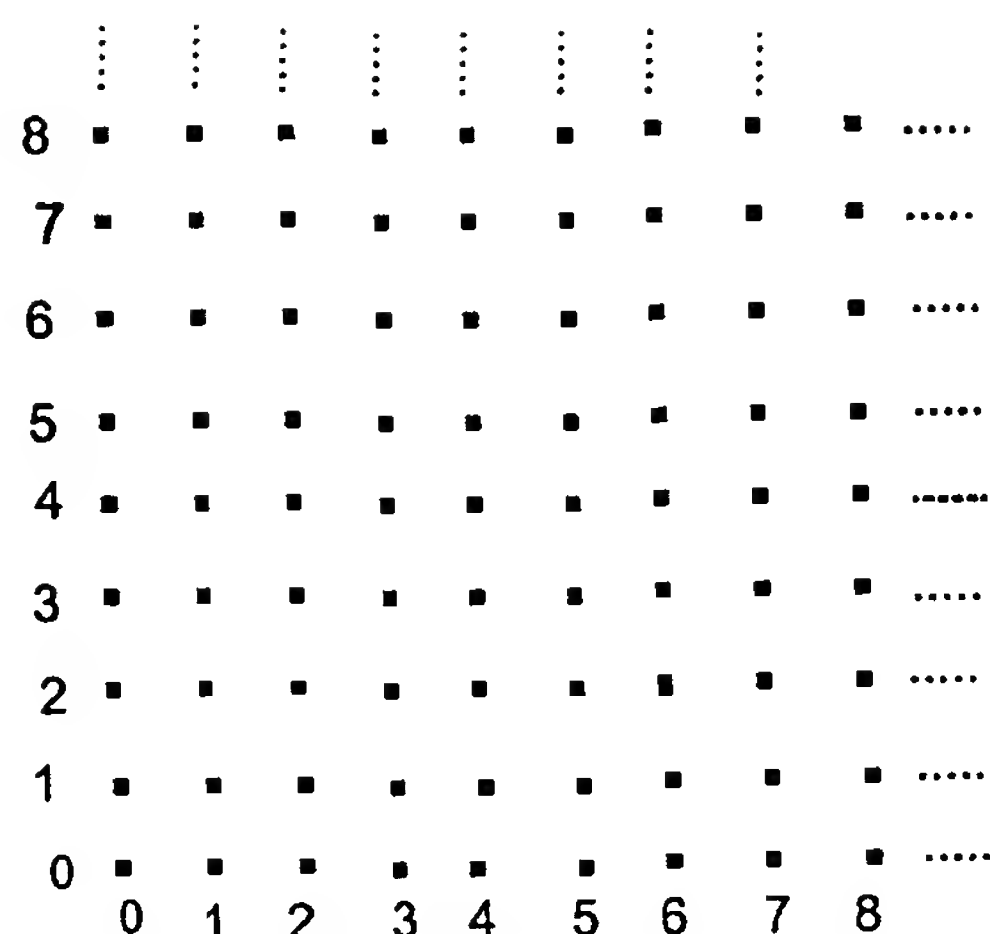
Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically innocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Orientation of the Linker

Different orientations can be achieved by including linkers containing mono- or polycyclic groups, including aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). Other groups can also include oligomers and polymers which are branched- or straight-chain species.

Different linkers can be designed to provide preferred orientations of the ligands. Such linkers or frameworks may be represented by using an array of dots (as shown below) wherein each dot may potentially be an atom, such as C, O, N, S, P, H, F, Cl, Br, and F or the dot may alternatively indicate the absence of an atom at that position. To facilitate the understanding of the framework structure, the framework is illustrated as a two dimensional array in the following diagram, although clearly the framework is a three dimensional array in practice:

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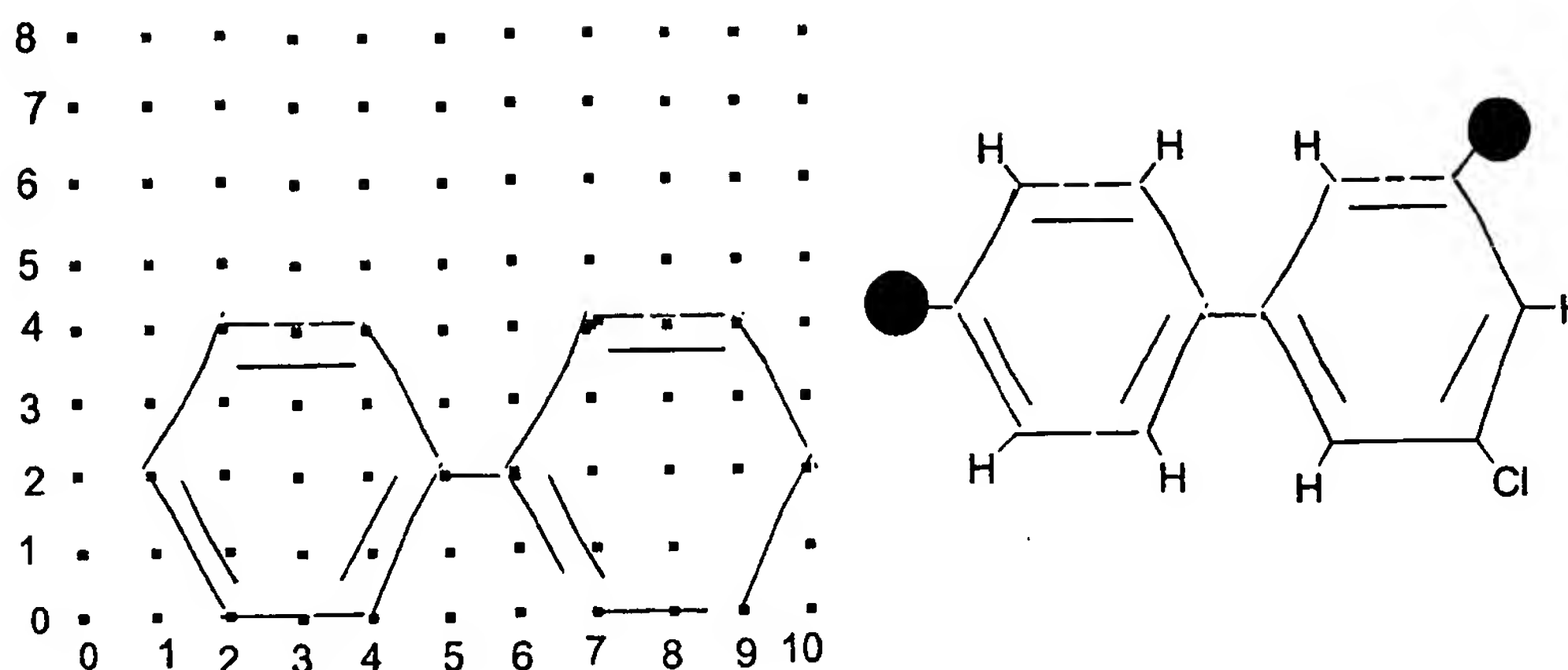


Each dot is either an atom, chosen from carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, or halogen, or the dot represents a point in space (i.e., an absence of an atom). As is apparent to the skilled artisan, only certain atoms on the grid have the ability to act as an attachment point for the ligands, namely, C, O, N, S and P.

Atoms can be connected to each other via bonds (single, double or triple bonds with acceptable resonance and tautomeric forms), with regard to the usual constraints of chemical bonding. Ligands may be attached to the framework via single, double or triple bonds (with chemically acceptable tautomeric and resonance forms). Multiple ligand groups (2 to 10) can be attached to the framework such that the minimal, shortest path distance between adjacent ligand groups does not exceed 100 atoms. Preferably, the linker connections to the ligand is selected such that the maximum spatial distance between two adjacent ligands is no more than 40Å.

An example of a linker as presented by the grid is shown below for a biphenyl construct.

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Nodes (1,2), (2,0), (4,4), (5,2), (4,0), (6,2), (7,4), (9,4), (10,2), (9,0), (7,0) all represent carbon atoms. Node (10,0) represents a chlorine atom. All other nodes (or dots) are points in space (i.e., represent an absence of atoms).

5

Nodes (1,2) and (9,4) are attachment points. Hydrogen atoms are affixed to nodes (2,4), (4,4), (4,0), (2,0), (7,4), (10,2) and (7,0). Nodes (5,2) and (6,2) are connected by a single bond.

10

The carbon atoms present are connected by either a single, double or triple bonds, taking into consideration the principle of resonance and/or tautomerism.

15

The intersection of the framework (linker) and the ligand group, and indeed, the framework (linker) itself can have many different bonding patterns. Examples of acceptable patterns of three contiguous atom arrangements are shown in the following diagram:

20

CCC	NCC	OCC	SCC	PCC
CCN	NCN	OCN	SCN	PCN
CCO	NCO	OCO	SCO	PCO
CCS	NCS	OCS	SCS	PCS
CCP	NCP	OCP	SCP	PCP

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5	CNC	NNC	ONC	SNC	PNC
	CNN	NNN	ONN	<u>SNN</u>	PNN
	CNO	NNO	<u>ONO</u>	SNO	PNO
	CNS	<u>NNS</u>	ONS	SNS	PNS
	CNP	<u>NNP</u>	ONP	SNP	PNP
10	COC	NOC	<u>OOC</u>	SOC	POC
	CON	<u>NON</u>	<u>ONN</u>	SON	PON
	<u>COO</u>	<u>NOO</u>	<u>OOO</u>	<u>SOO</u>	<u>POO</u>
	COS	<u>NOS</u>	<u>OOS</u>	<u>SOS</u>	<u>POS</u>
	COP	<u>NOP</u>	<u>OOP</u>	<u>SOP</u>	<u>POP</u>
15	CSC	NSC	OSC	SSC	PSC
	CSN	NSN	OSN	SSN	<u>PSN</u>
	CSO	NSO	OSO	<u>SSO</u>	<u>PSO</u>
	CSS	NSS	OSS	<u>SSS</u>	<u>PSS</u>
	CSP	<u>NSP</u>	<u>OSP</u>	<u>SSP</u>	<u>PSP</u>
20	CPC	NPC	OPC	SPC	<u>PPC</u>
	CPN	NPN	OPN	SPN	<u>PPN</u>
	CPO	NPO	OPO	SPO	<u>PPO</u>
	CPS	NPS	OPS	SPS	<u>PPS</u>
	<u>CPP</u>	<u>NPP</u>	<u>OPP</u>	<u>SPP</u>	<u>PPP</u>

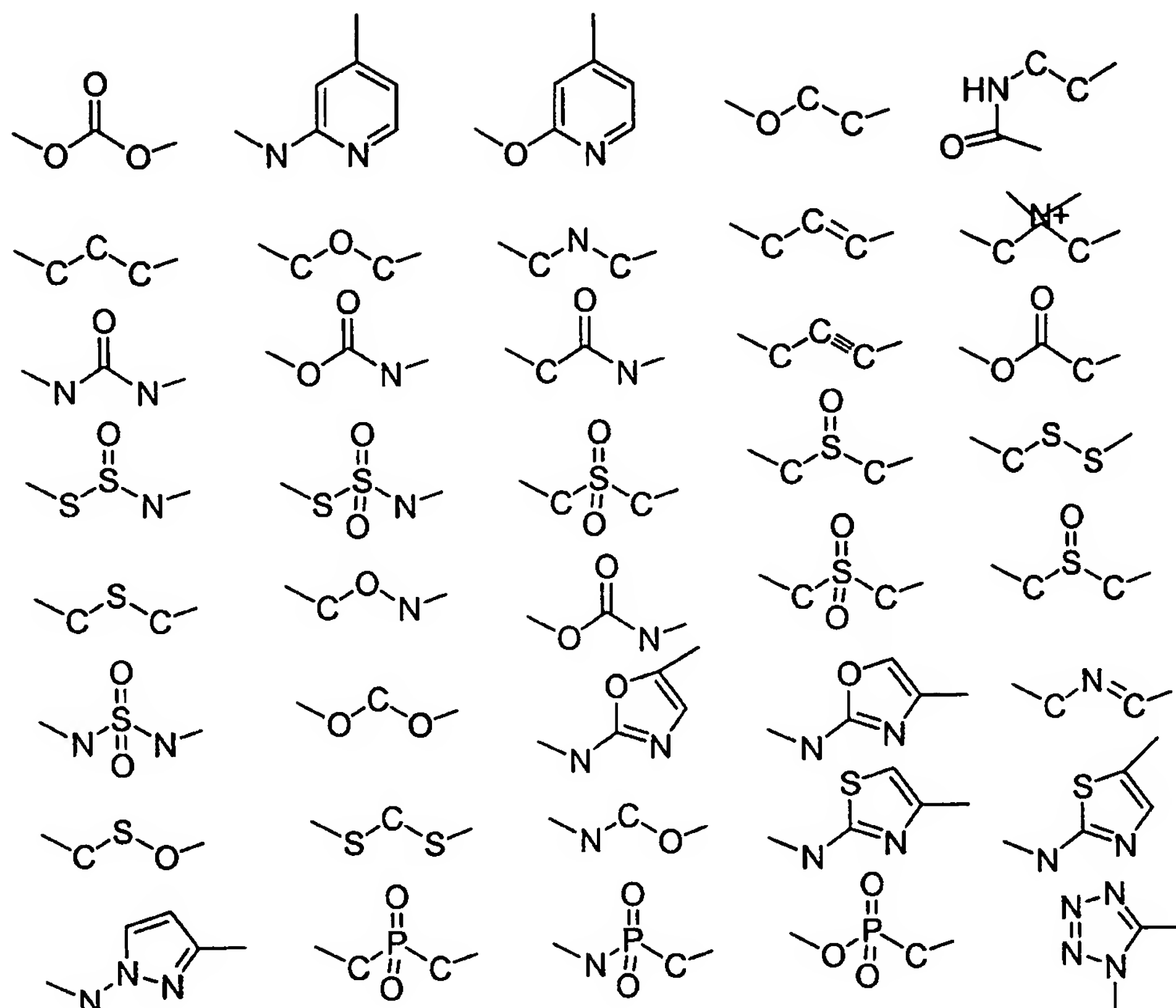
25 One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in March, "Advanced Organic Chemistry", 4th Edition, Wiley-Interscience, New York, New York (1992). These arrangements are described in the grid of dots shown in the scheme above. All of the possible arrangements for the five most preferred

30 atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.

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The identification of an appropriate framework geometry and size for ligand domain presentation are important steps in the construction of a multibinding compound with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of appropriate frameworks through an iterative process. Figure 1 illustrates a useful strategy for determining an optimal framework display orientation for ligand domains. Various other strategies are known to those skilled in the art of molecular design and can be used for preparing compounds of this invention.

As shown in Figure 1, display vectors around similar central core structures such as a phenyl structure and a cyclohexane structure can be varied, as can the spacing of the ligand domain from the core structure (i.e., the length of the attaching moiety). It is to be noted that core structures other than those shown here can be used for determining the optimal framework display orientation of the ligands. The process may require the use of

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multiple copies of the same central core structure or combinations of different types of display cores.

The above-described process can be extended to trimers (Figure 2) and compounds of higher valency. In Figure 1 and 2, the shaded circle represents the ligand attached to the particular linkers.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available (such as 7TM G-protein coupled receptors), one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two

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binding sites reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, polarizability, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

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Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

5

It is understood that one or more of the above described properties of the linker can be modified by methods well known in the art. For example, the addition or insertion of ancillary groups into or onto the linker is known to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto or into the linker enhances the hydrophilicity and water solubility of the multibinding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

10
15

Ancillary groups which enhance the water solubility/hydrophilicity of the linker and, accordingly, the resulting multibinding compounds are also known in the art. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligosaccharides, etc.), carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like) to enhance the water solubility and/or hydrophilicity of the multibinding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether .

20
25

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the multibinding compounds described herein is also known in the art. Lipophilic groups useful with the linkers of this invention include, by way of example only, aryl and heteroaryl groups which, as above,

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may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives which do not form bilayers in aqueous medium until higher concentrations are reached.

5

Also known in the art is the use of ancillary groups which result in the multibinding compound being incorporated or anchored into a vesicle or other membranous structure such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or a micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

25

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker or bonds between the linker and the ancillary group(s) or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the

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presence of rings and/or multiple bonds within the group, for example, aryl, heteroaryl, cycloalkyl, cycloalkenyl, and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

5 Rigidity may also be imparted by internal hydrogen bonding or by hydrophobic collapse.

 Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic
10 groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

15

 In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In other preferred embodiments, the linker comprises one or more six-membered rings. In still further preferred
embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl.

20

 Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each
25 other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker into a conformation which allows bonding between the oppositely
30 charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge when deprotected, following addition to the linker, include

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deprotection of a carboxyl, hydroxyl, thiol or amino group by a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art which result in removal of the protecting group, is within the scope of this invention.

5 In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, restricted/unrestricted rotation, the desired degree of hydrophobicity/hydrophilicity, etc. is well within the skill of the art. Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention. In certain cases, the antigenicity of a multibinding compound
10 may be eliminated or reduced by use of groups such as, for example, poly(ethylene glycol).

Combinatorial synthesis

Having chosen a set of n ligands (n being determined by the sum of the number of
15 different attachment points for each ligand chosen) and m linkers by the process outlined above, a library of $(n!)m$ candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations
20 provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

25 When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalities on the ligands with complementary reactive
30 functionalities on the linkers. The library therefore lends itself to efficient parallel, e.g., split/pool, synthetic methods. The combinatorial library can employ solid phase

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chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial library is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

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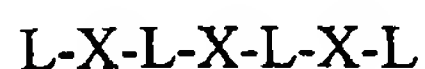
The multimeric compounds prepared in this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where each L is independently a ligand, each X is independently a linker, p is an integer from 2 to 10 and q is an integer from 1 to 20 and pharmaceutically acceptable salts thereof. These multimeric compounds are intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is described below.

10

As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position.

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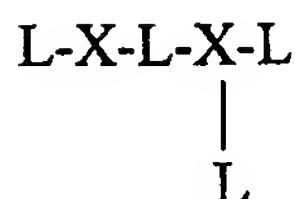
The simplest and most preferred multibinding compound is a bivalent compound which can be represented as L-X-L, where each L is independently a ligand which may be the same or different and each X is independently the linker. Examples of such bivalent compounds are provided in FIG. 1 where each shaded circle represents a ligand. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as can X. However, a trimer can also be a radial multibinding compound comprising three ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group. Illustrations of trivalent compounds prepared in this invention are found in FIG. 2 where, again, the shaded circles represent ligands. Tetravalent compounds can be represented in a linear array, e.g.,

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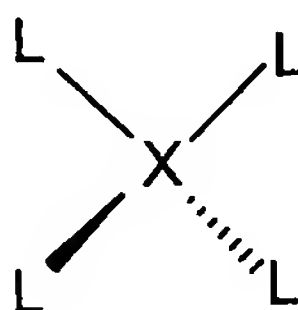
-63-

in a branched array, e.g.,

5



(a branched construct analogous to the isomers of butane -- *n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl) or in a tetrahedral array, e.g.,



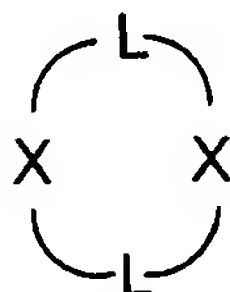
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where X and L are as defined herein. Alternatively, it could be represented as an alkyl, aryl or cycloalkyl derivative as above with four (4) ligands attached to the core linker.

The same considerations apply to higher multibinding compounds of this invention containing 5-10 ligands. However, for multibinding agents attached to a central linker such as aryl or cycloalkyl, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not directly accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

20

Certain of the above described compounds may alternatively be represented as cyclic chains of the form:



and variants thereof.

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All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$.

Analysis of array by biochemical, analytical, pharmacological, and computational
5 methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values can be determined. A combination of NMR
10 spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. *In vitro*
15 efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, can also be determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data can be determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future
20 work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described
25 above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing
30 determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et

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al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety.

Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998 which is incorporated herein by reference in its entirety. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates, diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case,

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the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

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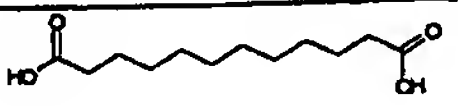
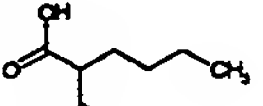
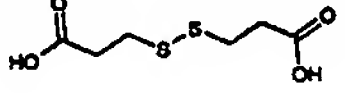

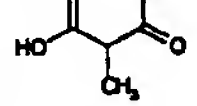
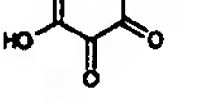
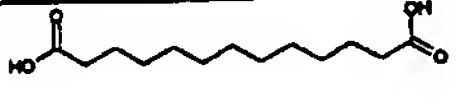
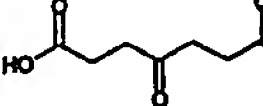
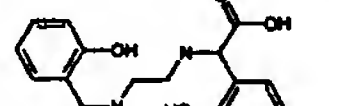
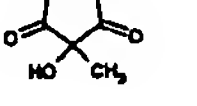
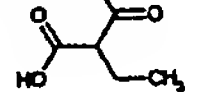
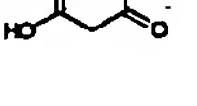
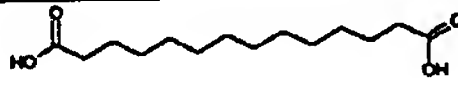
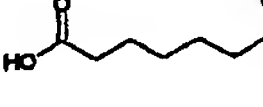

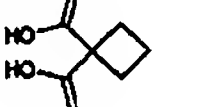
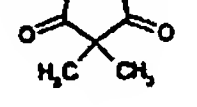
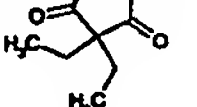
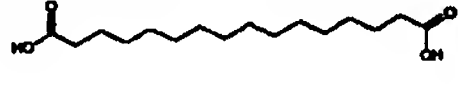
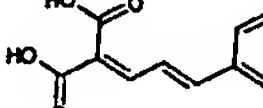


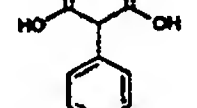
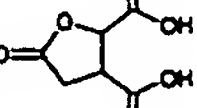
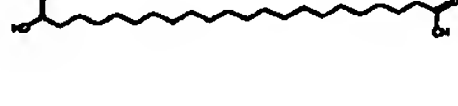
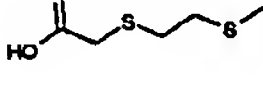
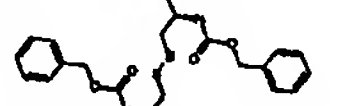
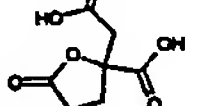
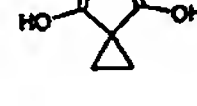
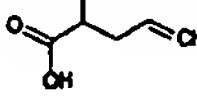
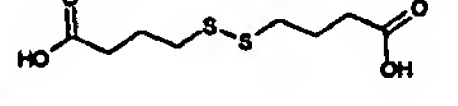
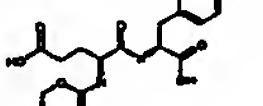
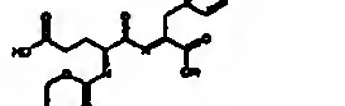
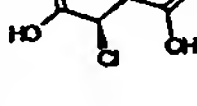
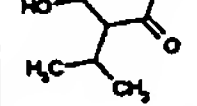



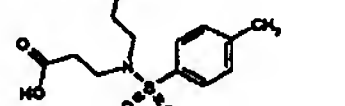

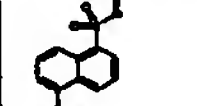
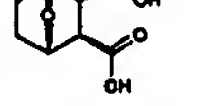
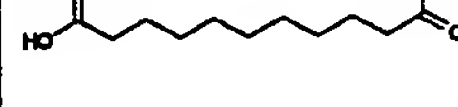
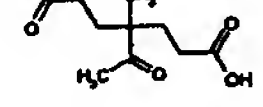
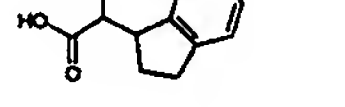
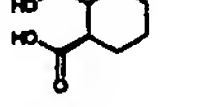

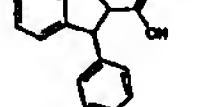
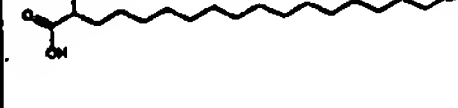
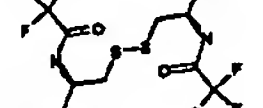
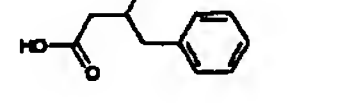
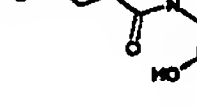
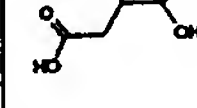
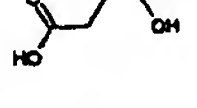
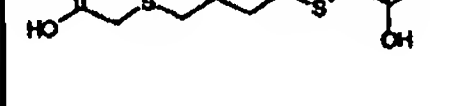

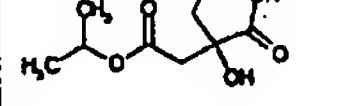
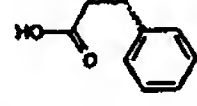
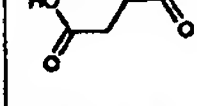
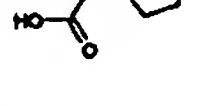

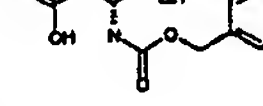
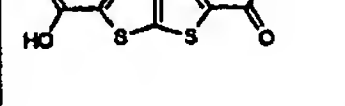
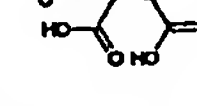

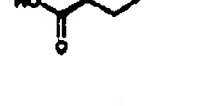

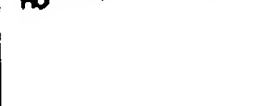

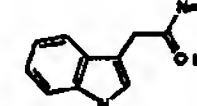
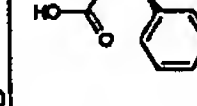

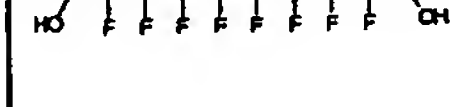


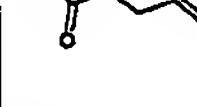
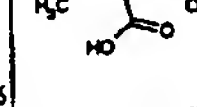
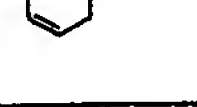

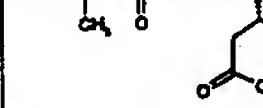

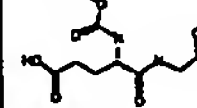

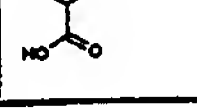
COMPLEMENTARY BINDING CHEMISTRIES

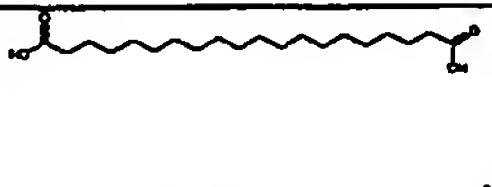
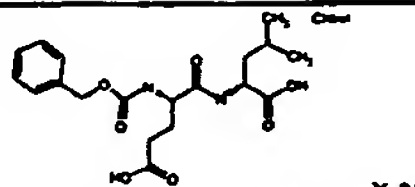
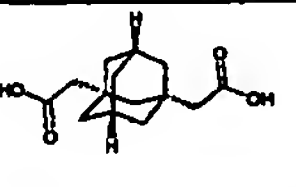
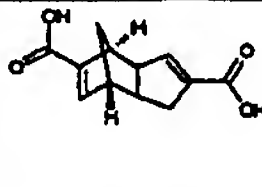
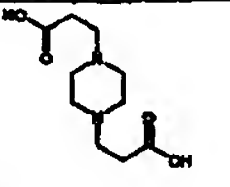
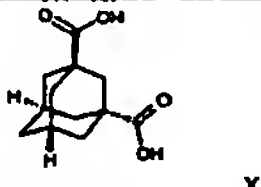
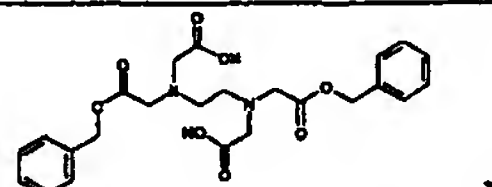
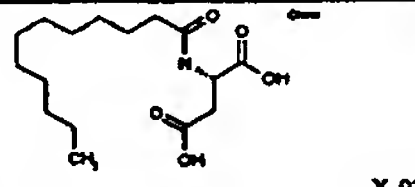
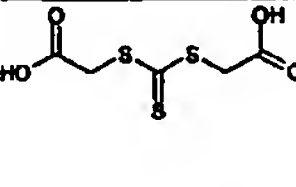
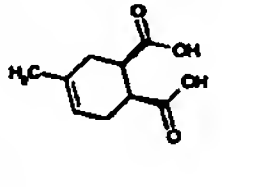
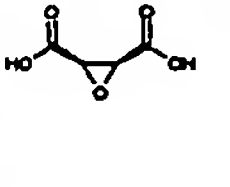
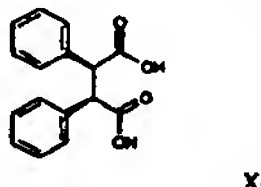
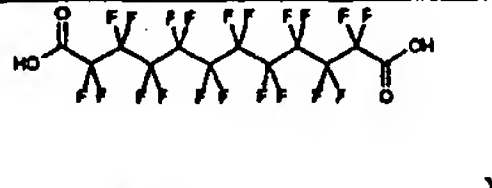
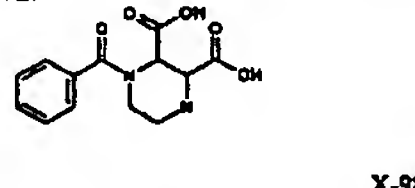
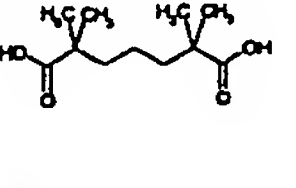
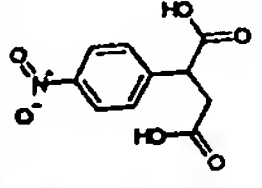
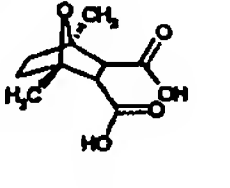
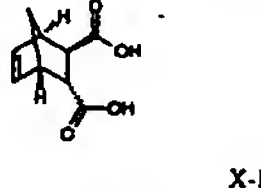
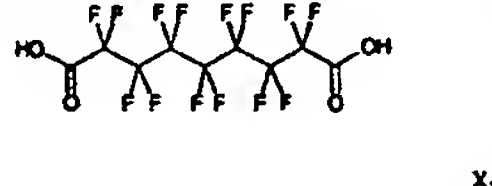
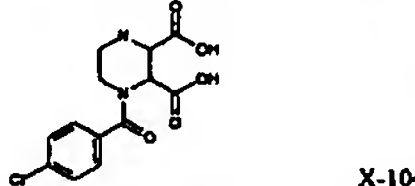
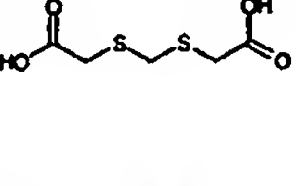
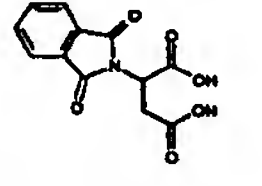
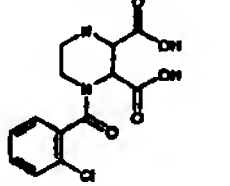
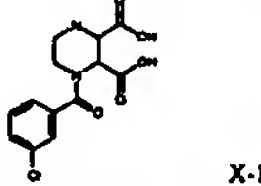
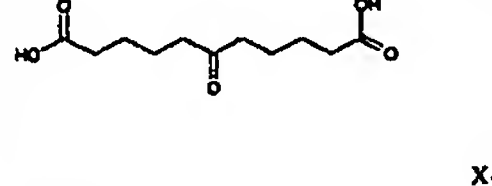
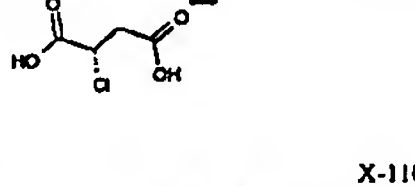
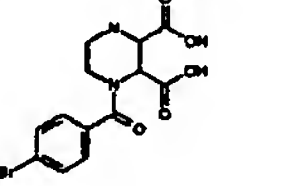
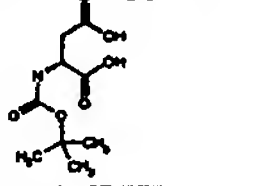
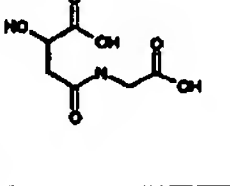
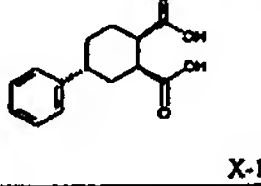
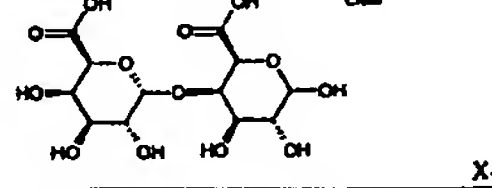
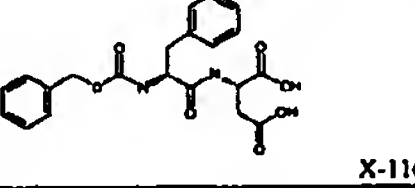
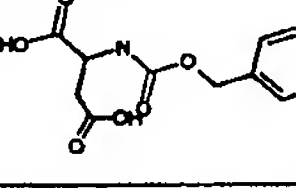
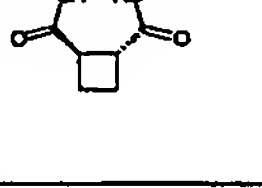
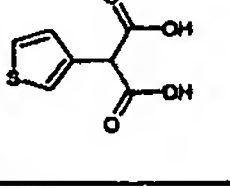
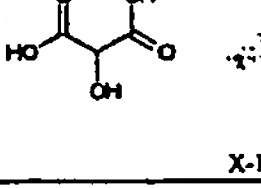
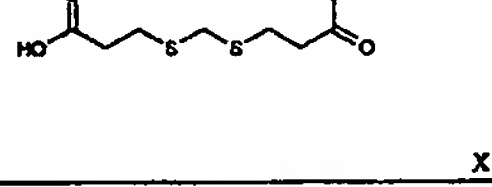
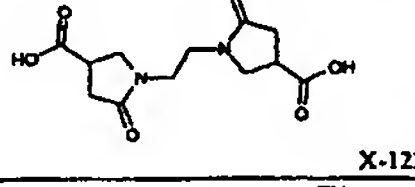
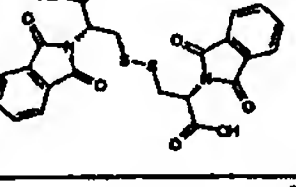
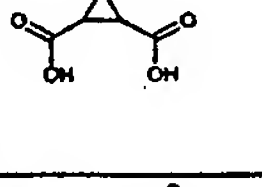
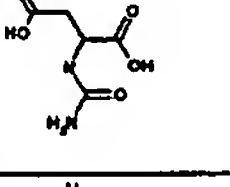
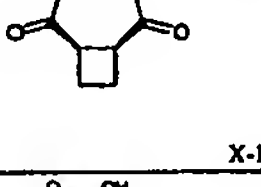
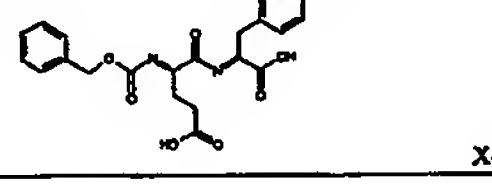
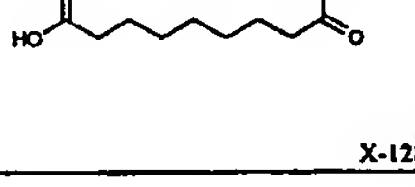
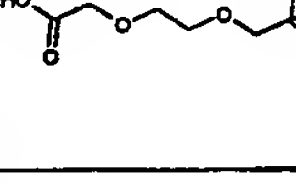
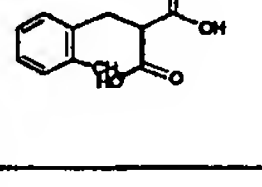
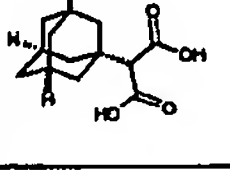
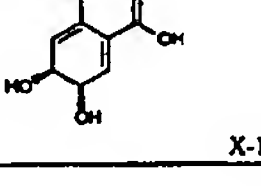
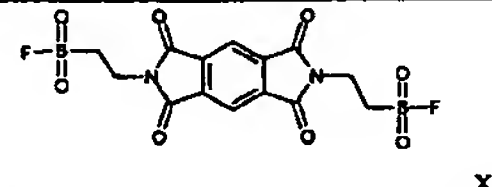
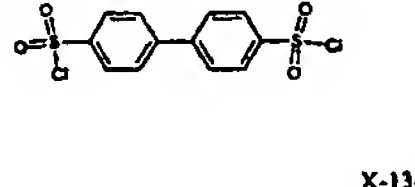
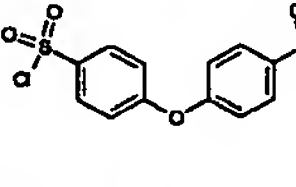
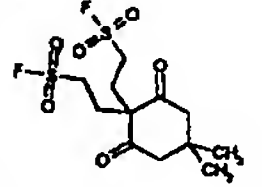
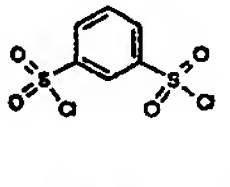
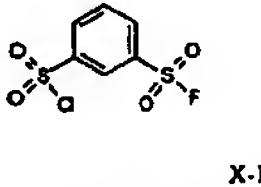
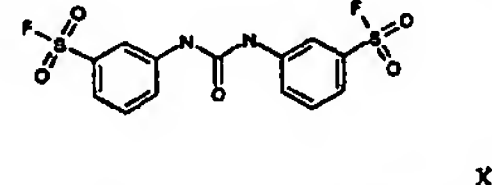
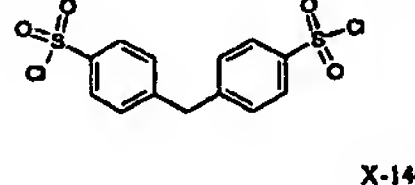
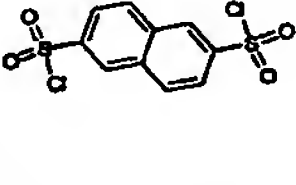
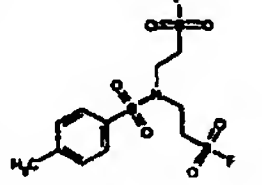
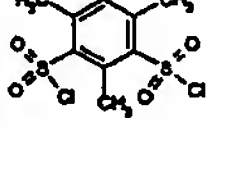
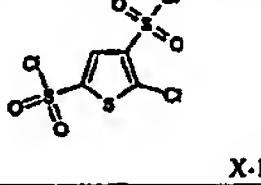
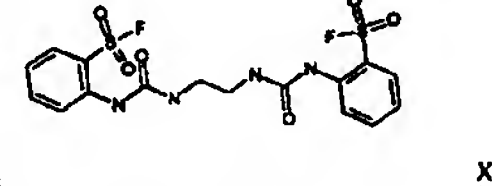
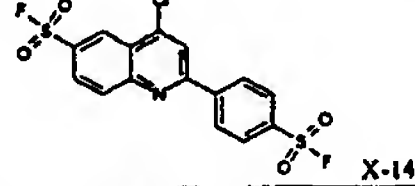
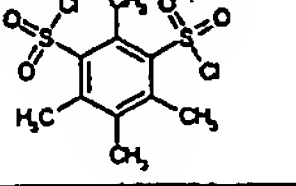
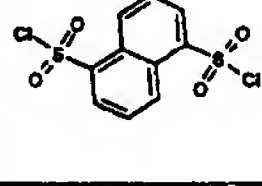
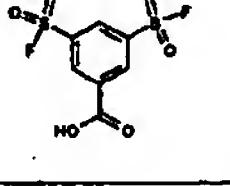
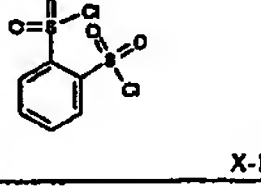
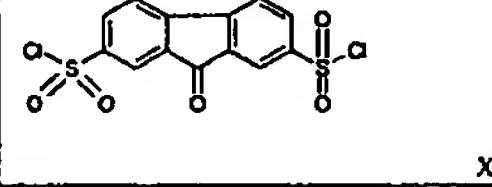
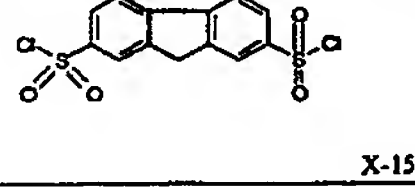
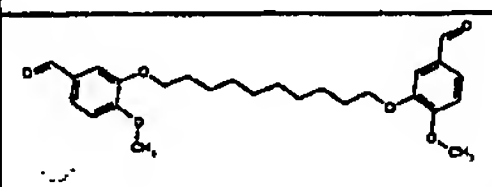
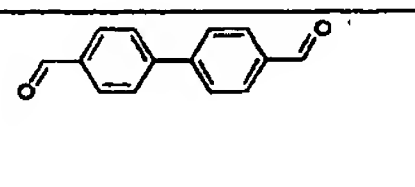
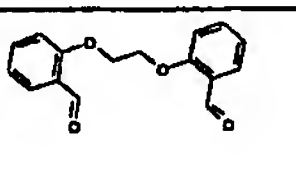
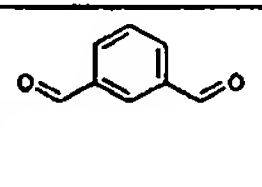
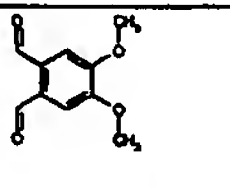
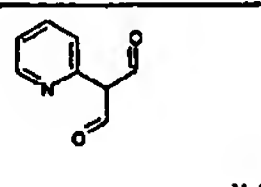
	<u>First Reactive Group</u>	<u>Second Reactive Group</u>	<u>Linkage</u>
10	hydroxyl amine sulfonyl halide carboxyl acid hydroxyl	isocyanate epoxide amine amine alkyl/aryl halide	urethane β -hydroxyamine sulfonamide amide ether
15	aldehyde ketone amine	amine/ NaCNBH_4 amine/ NaCNBH_4 isocyanate	amine amine urea

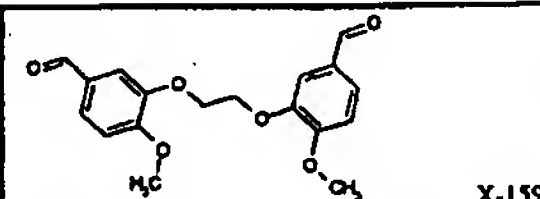
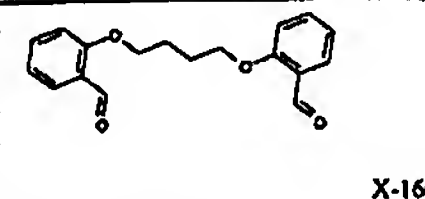
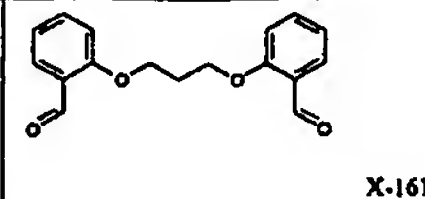
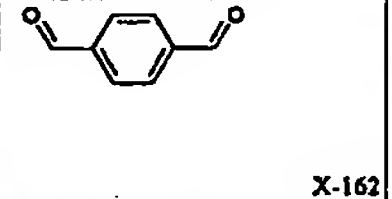
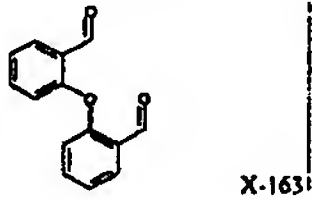
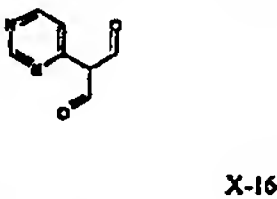
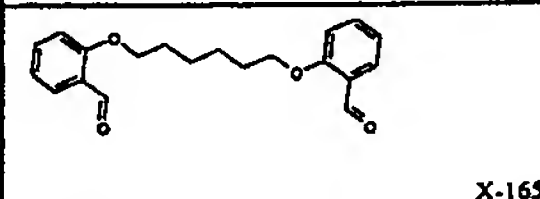
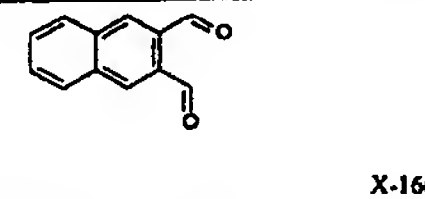
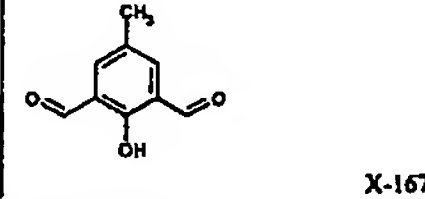
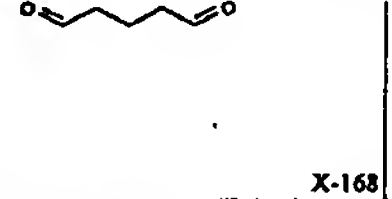
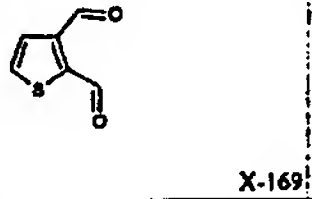
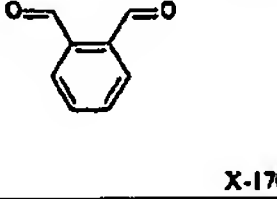
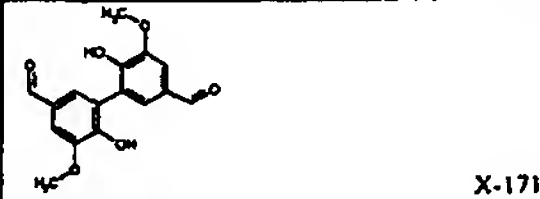
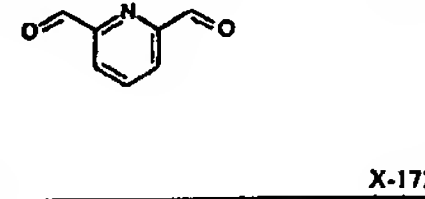
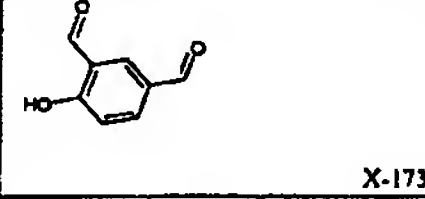
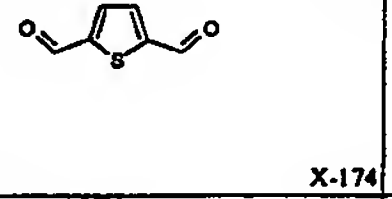
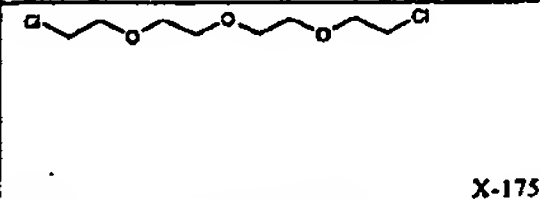
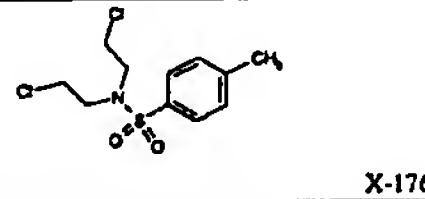
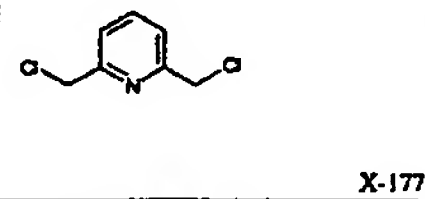
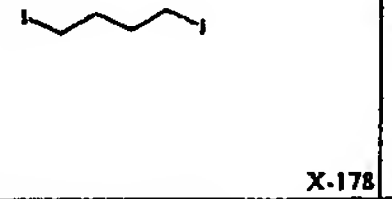
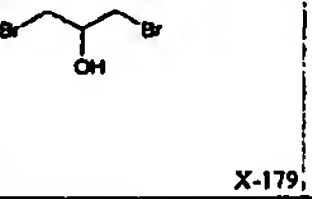
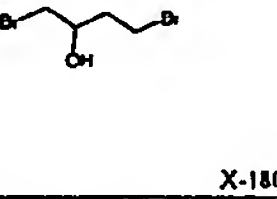
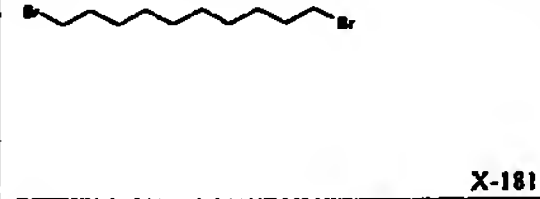
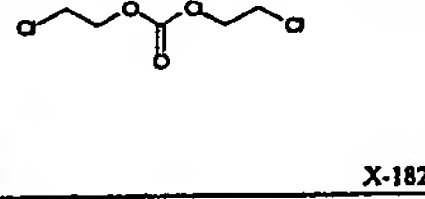
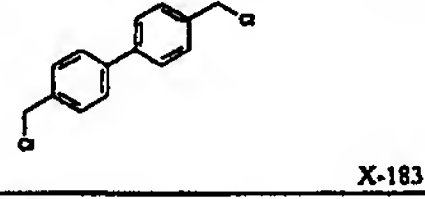
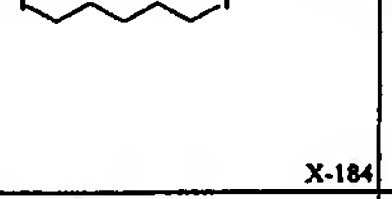
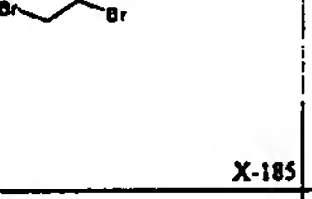
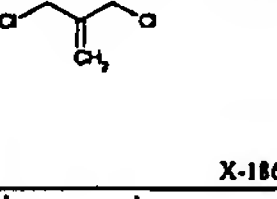
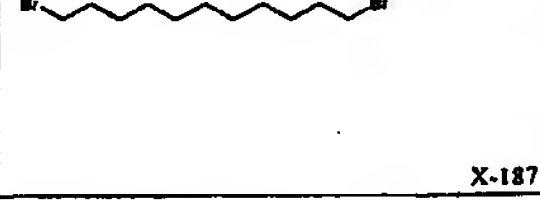
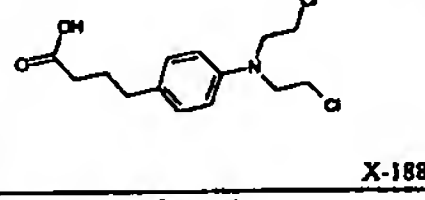
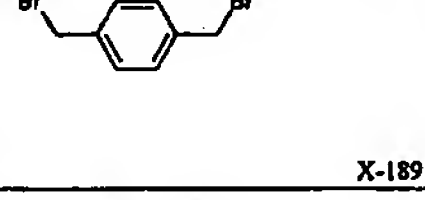
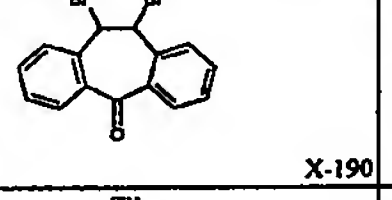
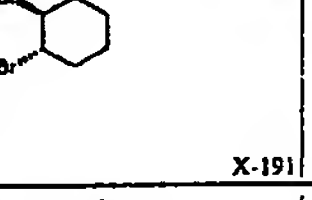
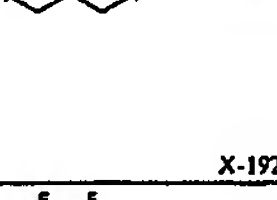
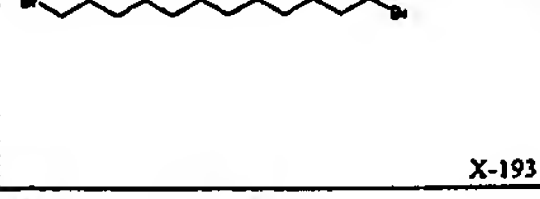
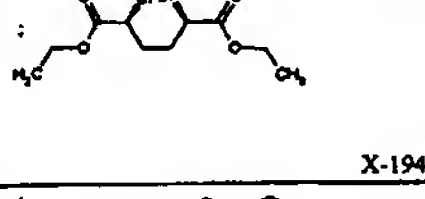
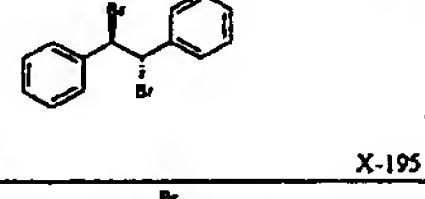
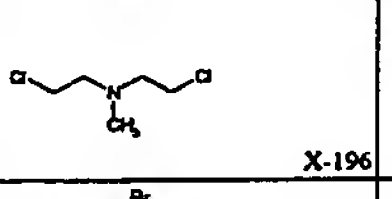
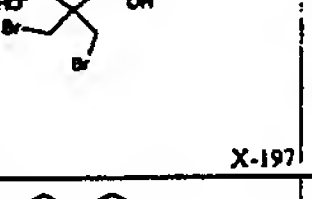
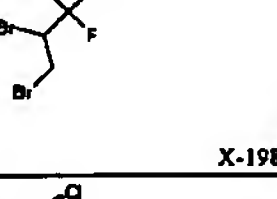
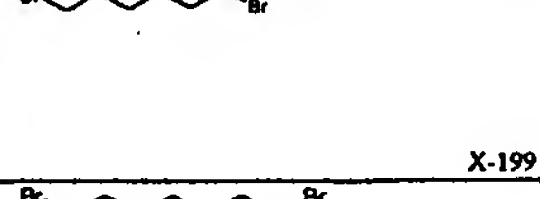
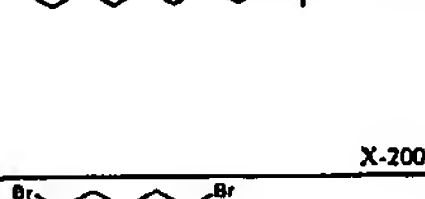
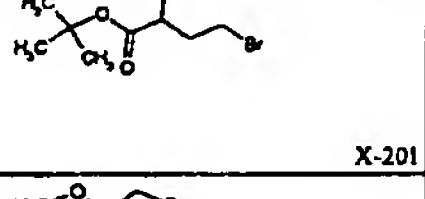
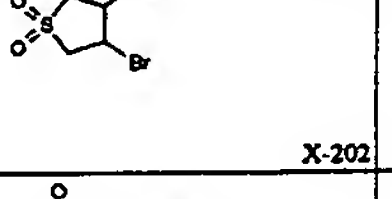
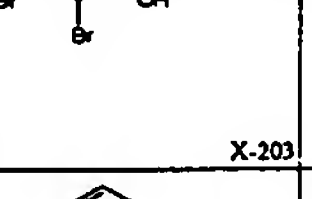
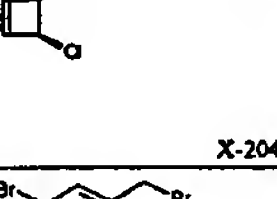


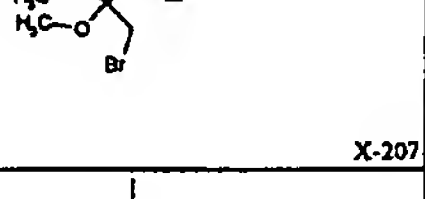
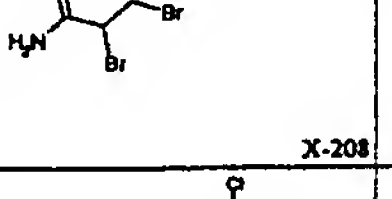
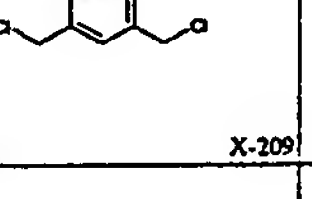
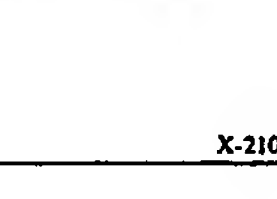
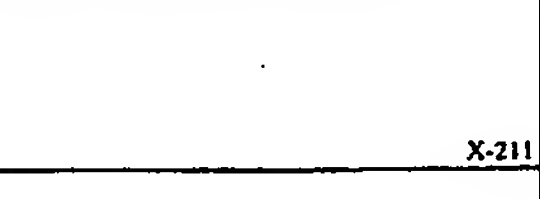
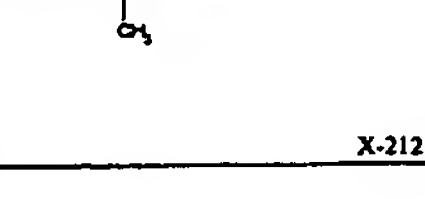
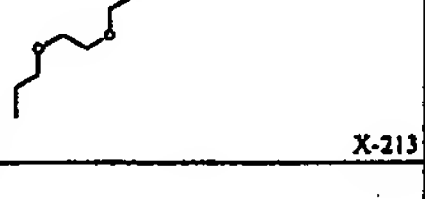
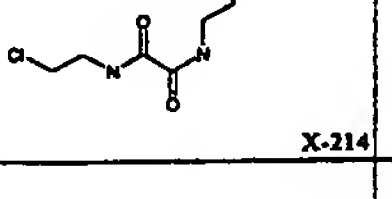
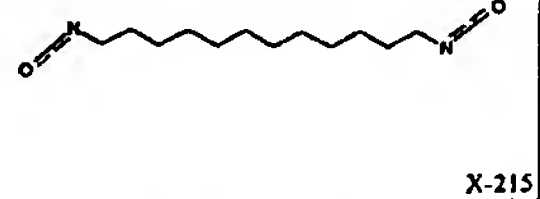
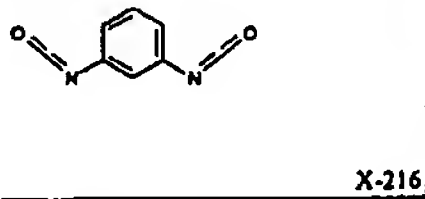
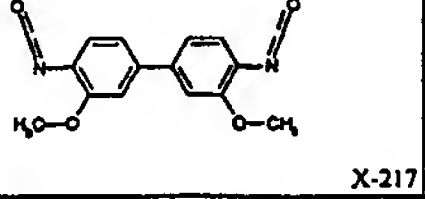
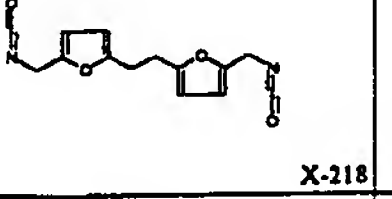
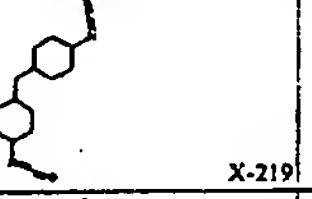
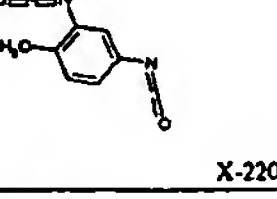
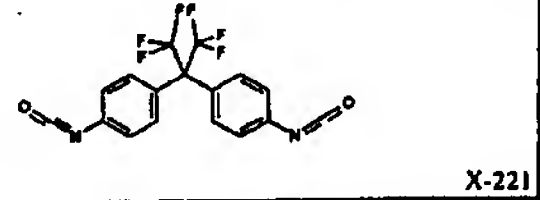
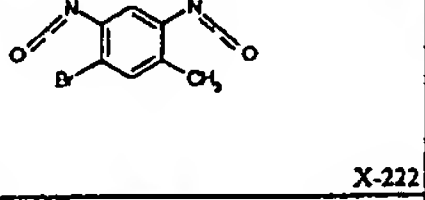
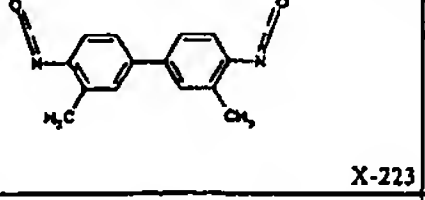
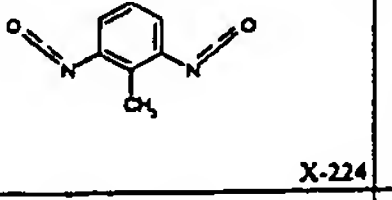
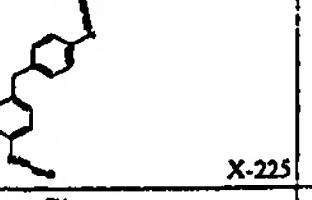
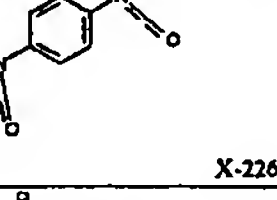

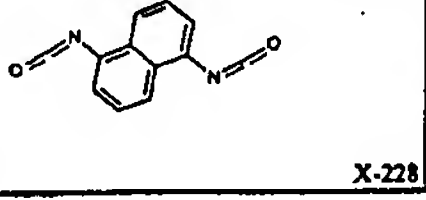
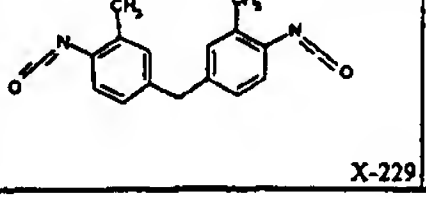
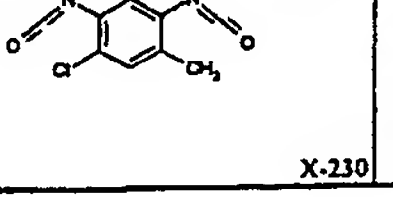
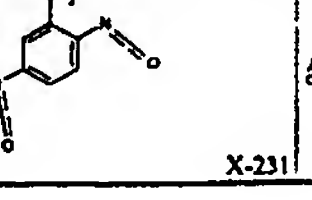
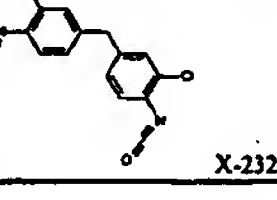
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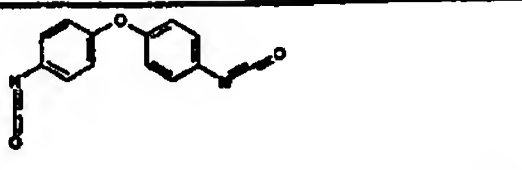
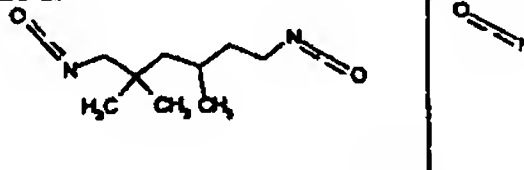
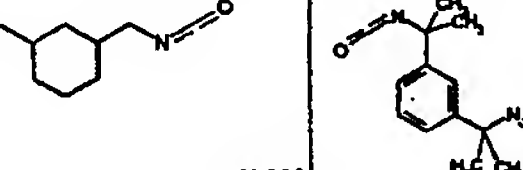
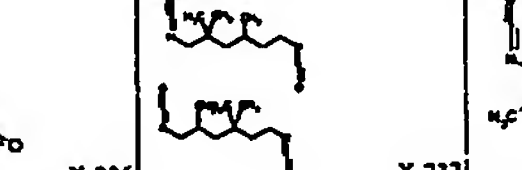


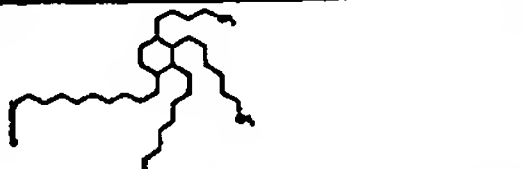
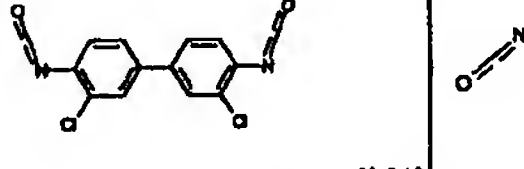

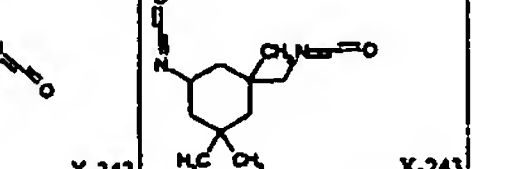

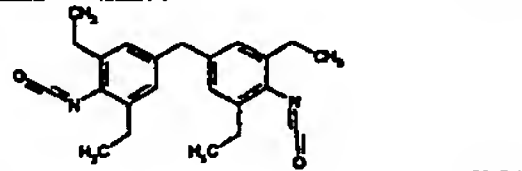
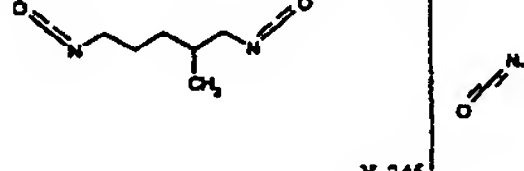
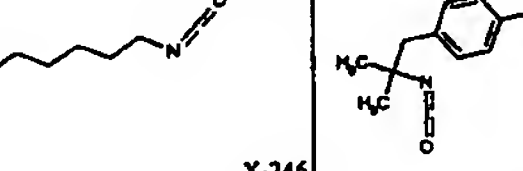
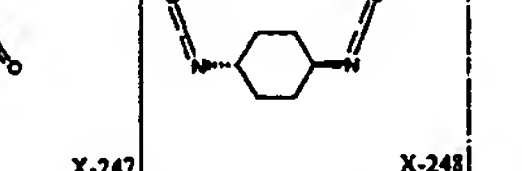

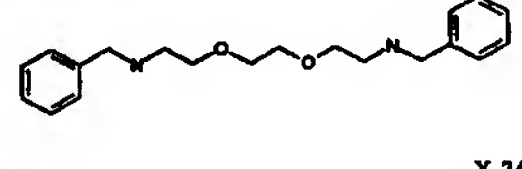
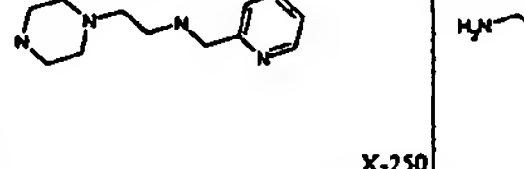
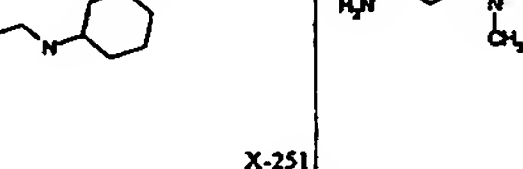
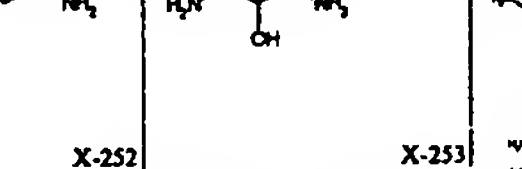


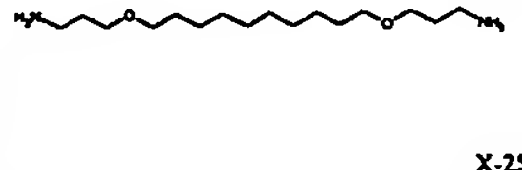
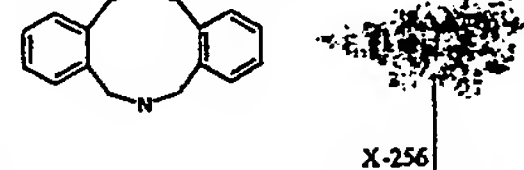
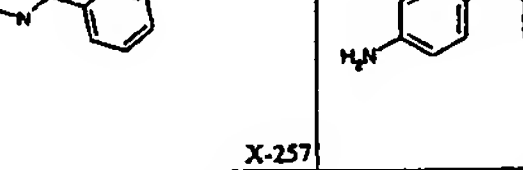
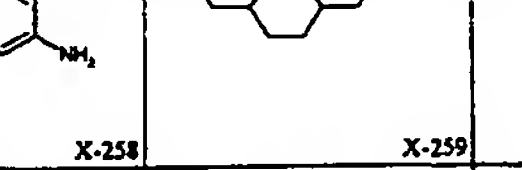
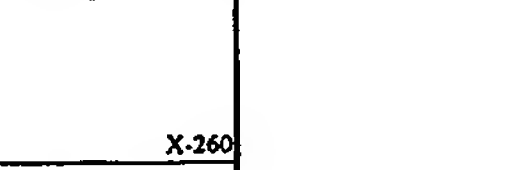

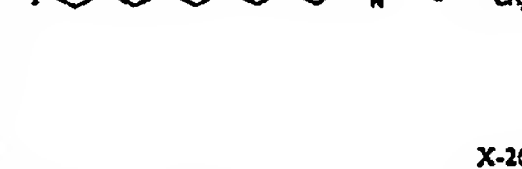
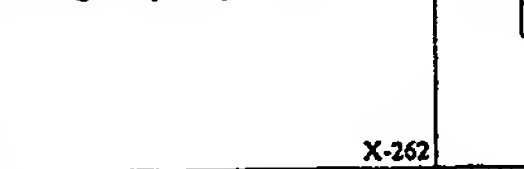
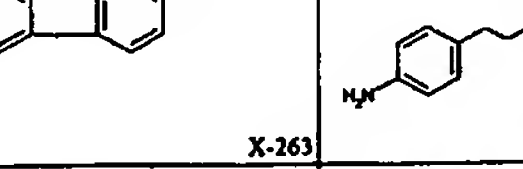

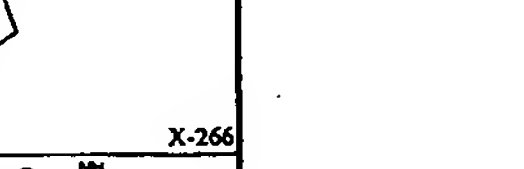

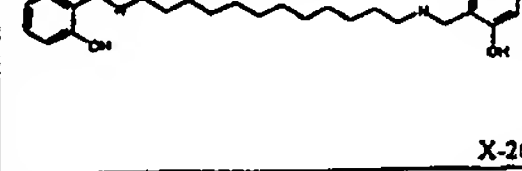
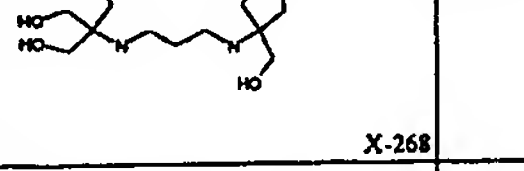
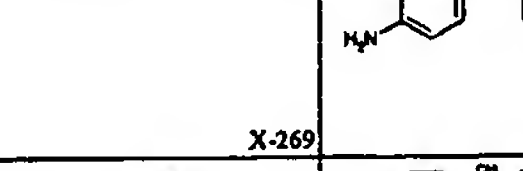
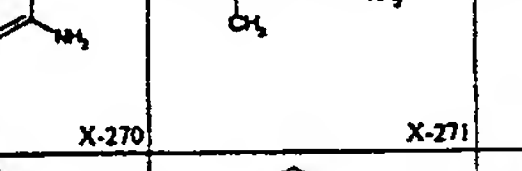
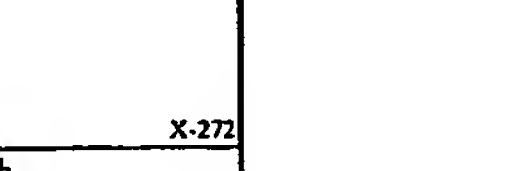

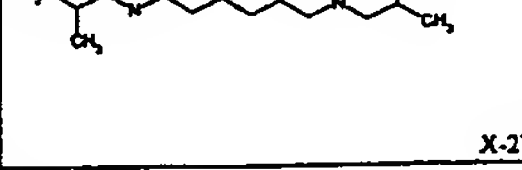
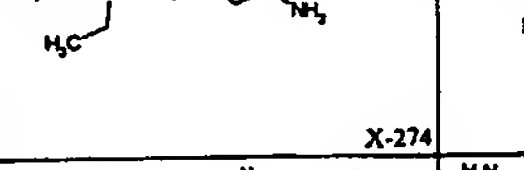
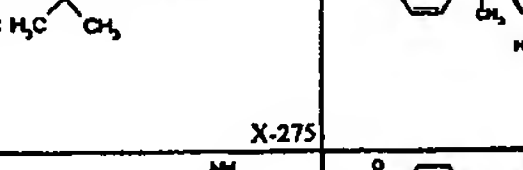
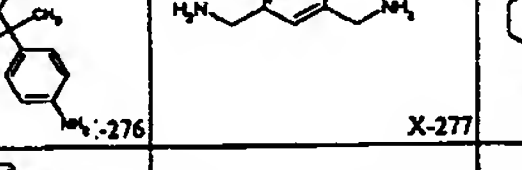
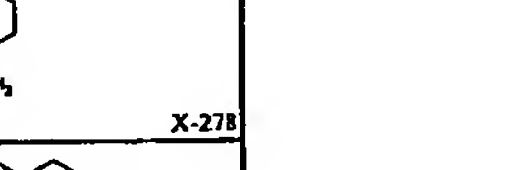



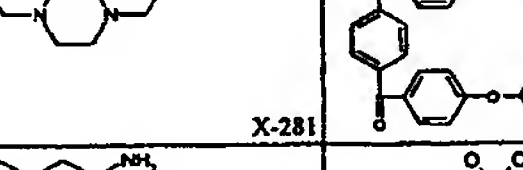
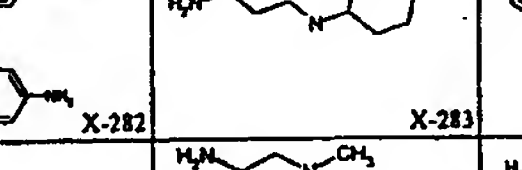
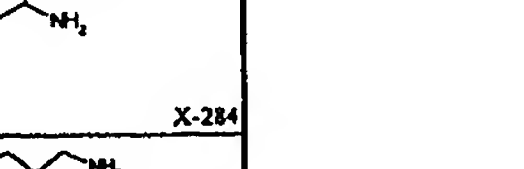

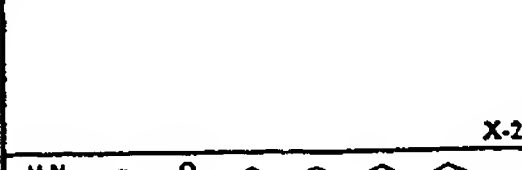

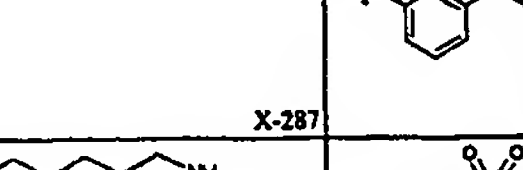

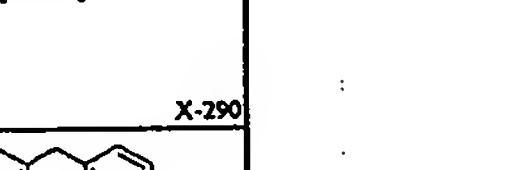


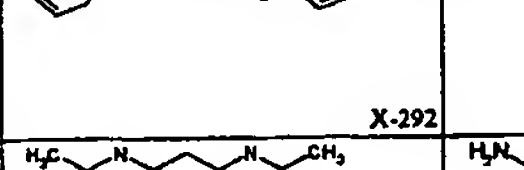
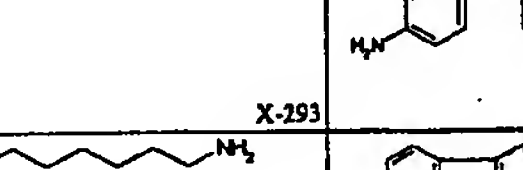

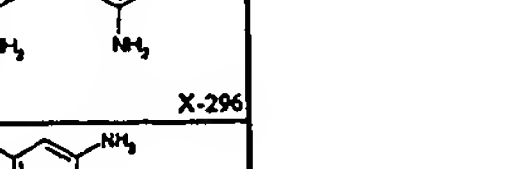

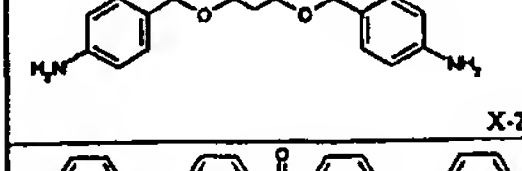
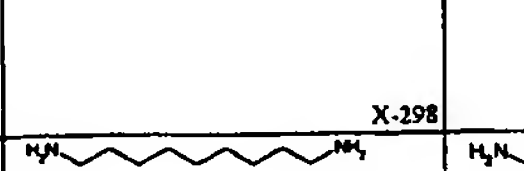
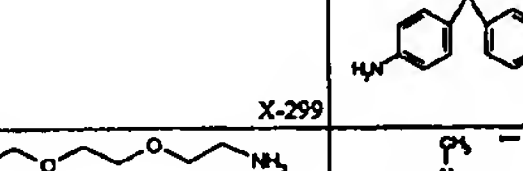
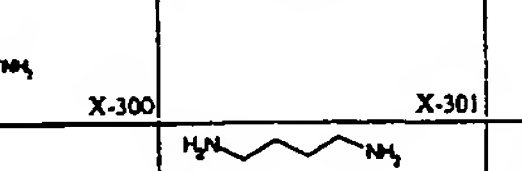
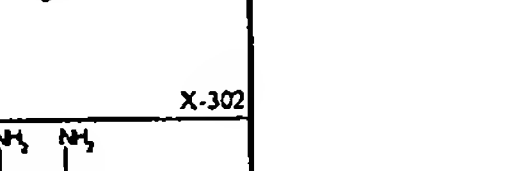



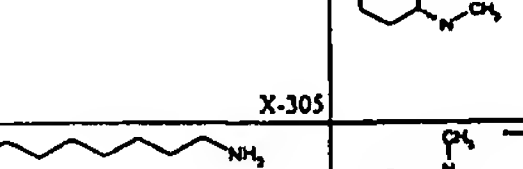
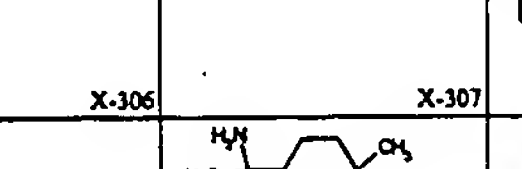
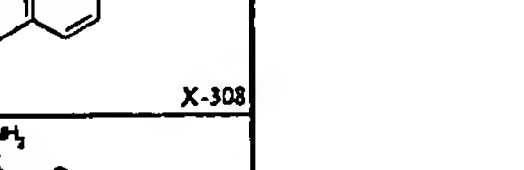

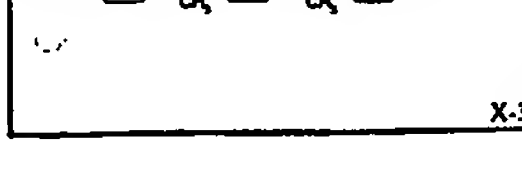
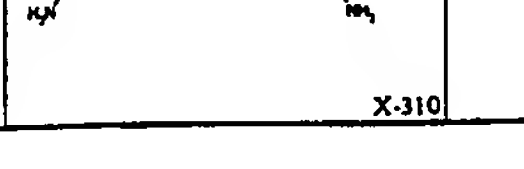
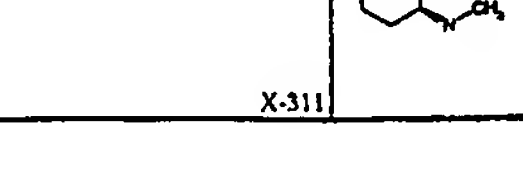
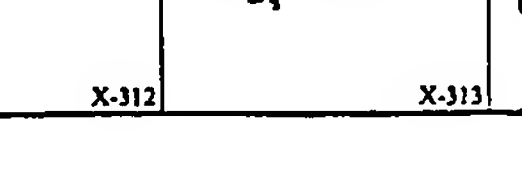
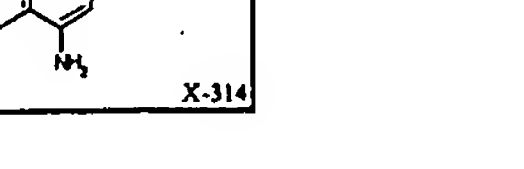

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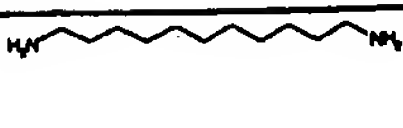
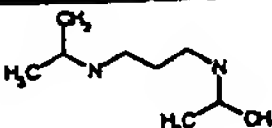
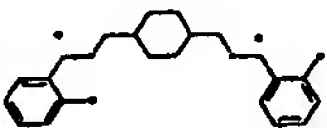
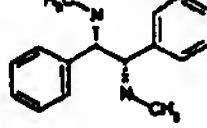
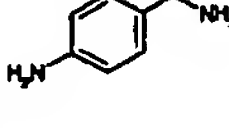
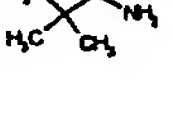





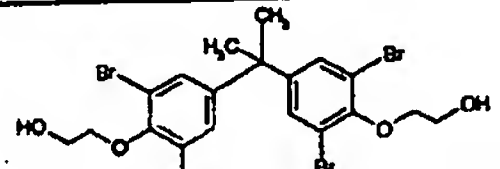

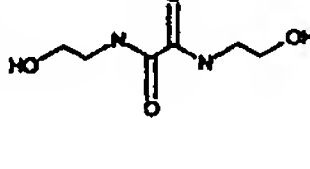
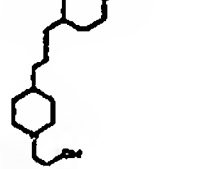
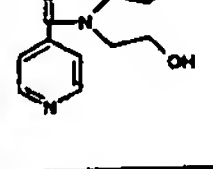

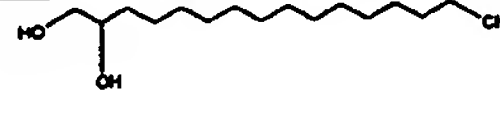
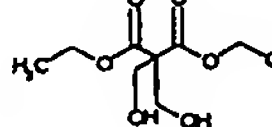
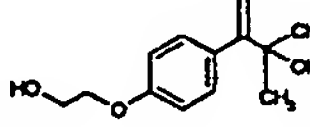
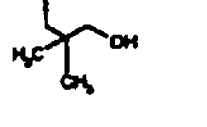
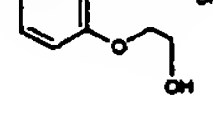
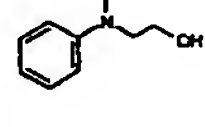


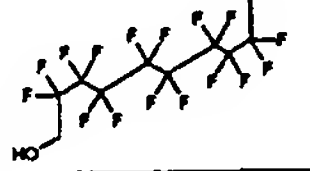
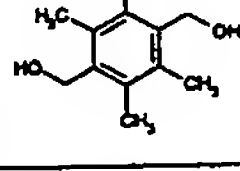
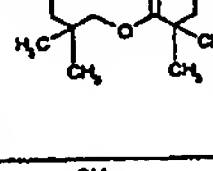
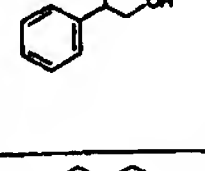


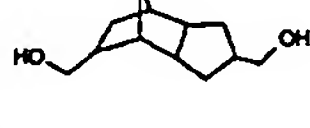
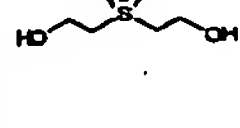
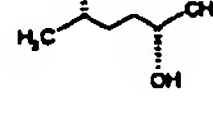





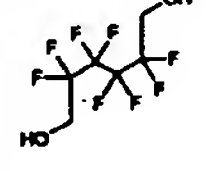
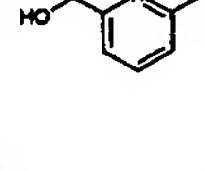
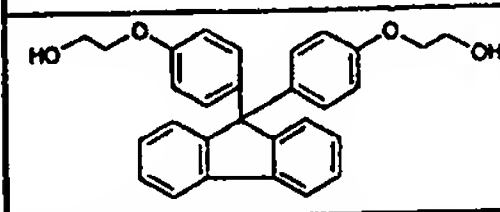






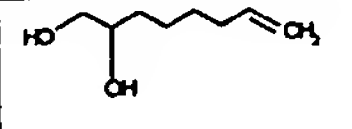
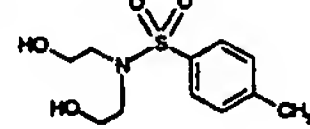




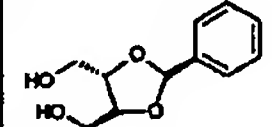


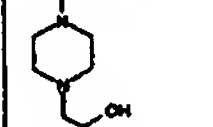
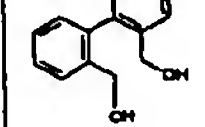

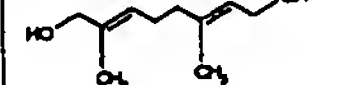

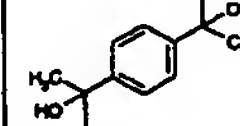


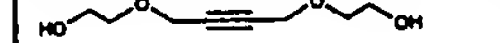











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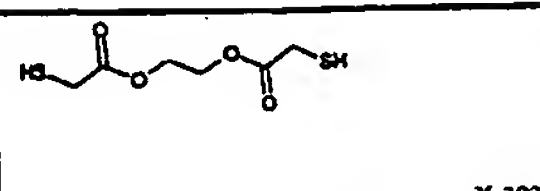
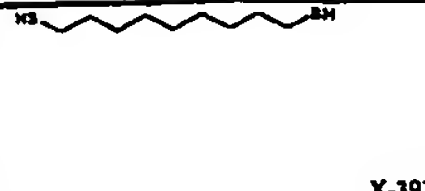
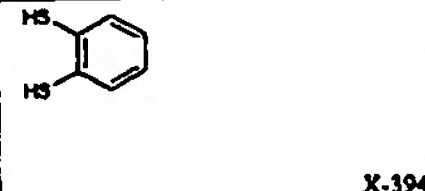
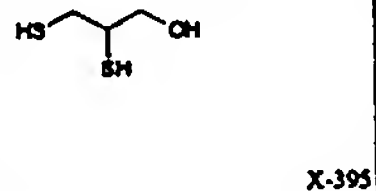
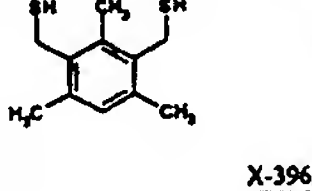
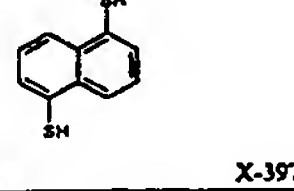
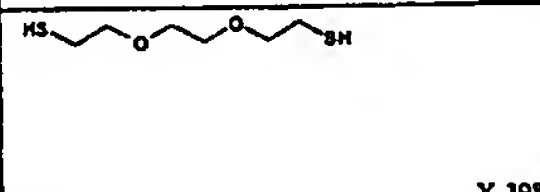
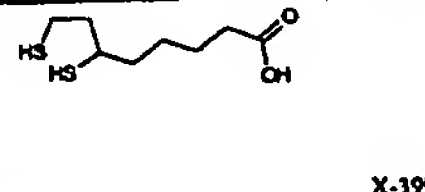
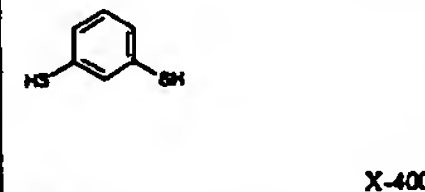
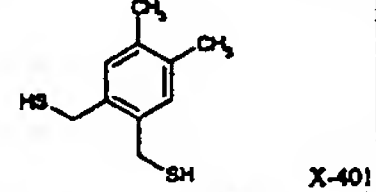
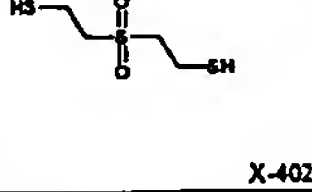
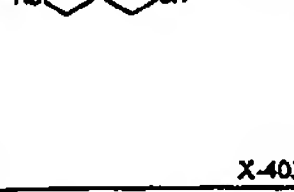
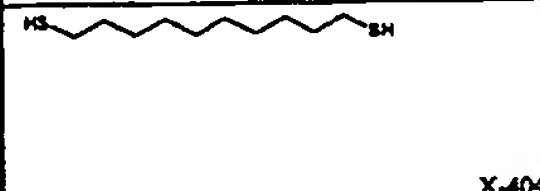
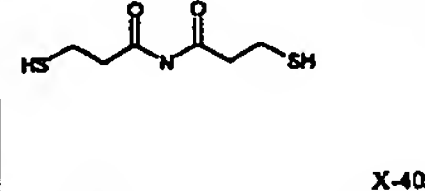
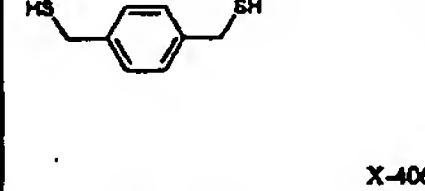
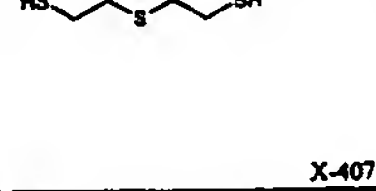
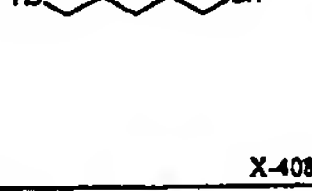
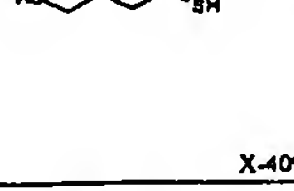
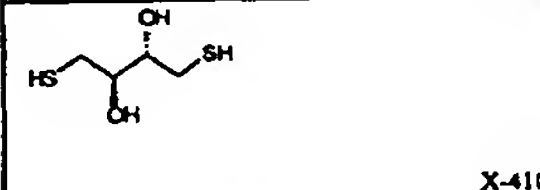
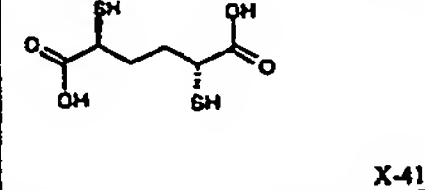
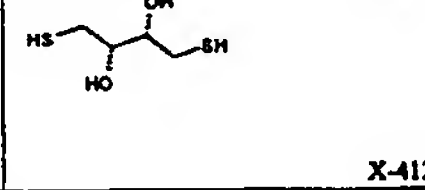
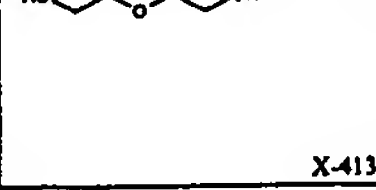
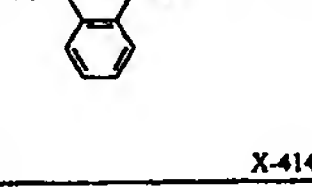
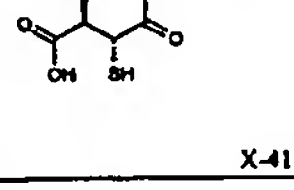
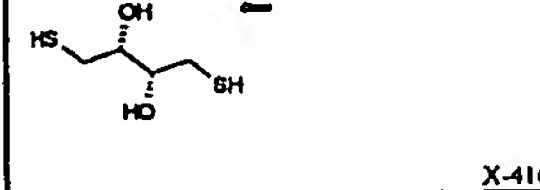
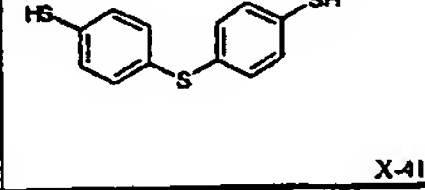
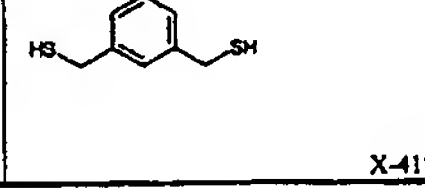
Diacids					
					
X-1	X-2	X-3	X-4	X-5	X-6
					
X-7	X-8	X-9	X-10	X-11	X-12
					
X-13	X-14	X-15	X-16	X-17	X-18
					
X-19	X-20	X-21	X-22	X-23	X-24
					
X-25	X-26	X-27	X-28	X-29	X-30
					
X-31	X-32	X-33	X-34	X-35	X-36
					
X-37	X-38	X-39	X-40	X-41	X-42
					
X-43	X-44	X-45	X-46	X-47	X-48
					
X-49	X-50	X-51	X-52	X-53	X-54
					
X-55	X-56	X-57	X-58	X-59	X-60
					
X-61	X-62	X-63	X-64	X-65	X-66
					
X-67	X-68	X-69	X-70	X-71	X-72
					
X-73	X-74	X-75	X-76	X-77	X-78
					
X-79	X-80	X-81	X-82	X-83	X-84

 X-85	 X-86	 X-87	 X-88	 X-89	 X-90
 X-91	 X-92	 X-93	 X-94	 X-95	 X-96
 X-97	 X-98	 X-99	 X-100	 X-101	 X-102
 X-103	 X-104	 X-105	 X-106	 X-107	 X-108
 X-109	 X-110	 X-111	 X-112	 X-113	 X-114
 X-115	 X-116	 X-117	 X-118	 X-119	 X-120
 X-121	 X-122	 X-123	 X-124	 X-125	 X-126
 X-127	 X-128	 X-129	 X-130	 X-131	 X-132
Disulfonyl Halides					
 X-133	 X-134	 X-135	 X-136	 X-137	 X-138
 X-139	 X-140	 X-141	 X-142	 X-143	 X-144
 X-145	 X-146	 X-147	 X-148	 X-149	 X-150
 X-151	 X-152				
Dialdehydes					
 X-153	 X-154	 X-155	 X-156	 X-157	 X-158

 X-159	 X-160	 X-161	 X-162	 X-163	 X-164
 X-165	 X-166	 X-167	 X-168	 X-169	 X-170
 X-171	 X-172	 X-173	 X-174		
Dihalides					
 X-175	 X-176	 X-177	 X-178	 X-179	 X-180
 X-181	 X-182	 X-183	 X-184	 X-185	 X-186
 X-187	 X-188	 X-189	 X-190	 X-191	 X-192
 X-193	 X-194	 X-195	 X-196	 X-197	 X-198
 X-199	 X-200	 X-201	 X-202	 X-203	 X-204
 X-205	 X-206	 X-207	 X-208	 X-209	 X-210
 X-211	 X-212	 X-213	 X-214		
Diisocyanates					
 X-215	 X-216	 X-217	 X-218	 X-219	 X-220
 X-221	 X-222	 X-223	 X-224	 X-225	 X-226
 X-227	 X-228	 X-229	 X-230	 X-231	 X-232

 X-233	 X-234	 X-235	 X-236	 X-237	 X-238
 X-239	 X-240	 X-241	 X-242	 X-243	
 X-244	 X-245	 X-246	 X-247	 X-248	
Diamines					
 X-249	 X-250	 X-251	 X-252	 X-253	 X-254
 X-255	 X-256	 X-257	 X-258	 X-259	 X-260
 X-261	 X-262	 X-263	 X-264	 X-265	 X-266
 X-267	 X-268	 X-269	 X-270	 X-271	 X-272
 X-273	 X-274	 X-275	 X-276	 X-277	 X-278
 X-279	 X-280	 X-281	 X-282	 X-283	 X-284
 X-285	 X-286	 X-287	 X-288	 X-289	 X-290
 X-291	 X-292	 X-293	 X-294	 X-295	 X-296
 X-297	 X-298	 X-299	 X-300	 X-301	 X-302
 X-303	 X-304	 X-305	 X-306	 X-307	 X-308
 X-309	 X-310	 X-311	 X-312	 X-313	 X-314

 X-315	 X-316	 X-317	 X-318	 X-319	 X-320
 X-321	 X-322	 X-323	 X-324	 X-325	
Diols					
 326	 X-327	 X-328	 X-329	 X-330	 X-331
 X-332	 X-333	 X-334	 X-335	 X-336	 X-337
 X-338	 X-339	 X-340	 X-341	 X-342	 X-343
 X-344	 X-345	 X-346	 X-347	 X-348	 X-349
 X-350	 X-351	 X-352	 X-353	 X-354	 X-355
 356	 X-357	 X-358	 X-359	 X-360	 X-361
 X-362	 X-363	 X-364	 X-365	 X-366	 X-367
 X-368	 X-369	 X-370	 X-371	 X-372	 X-373
 X-374	 X-375	 X-376	 X-377	 X-378	 X-379
 X-380	 X-381	 X-382	 X-383	 X-384	 X-385
Dithiols					
 X-386	 X-387	 X-388	 X-389	 X-390	 X-391

 X-392	 X-393	 X-394	 X-395	 X-396	 X-397
 X-398	 X-399	 X-400	 X-401	 X-402	 X-403
 X-404	 X-405	 X-406	 X-407	 X-408	 X-409
 X-410	 X-411	 X-412	 X-413	 X-414	 X-415
 X-416	 X-417	 X-418			

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In each case, any of the groups found on the linker including, by way of example only, acyl, alkyl, substituted alkyl, alkylene, substituted alkylene, alkynylene, substituted alkynylene, arylene (including optionally substituted arylene), cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, cycloalkylene, substituted cycloalkylene, heteroarylene (including optionally substituted heteroarylene, heterocyclene (including optionally substituted heterocyclene) groups can be replaced by any of these groups encompassed within the definitions for these substituents.

Representative ligands for use in this invention include, by way of example, L-1 through L-2 as identified below:

L-1 first ligand for muscarinic receptor (e.g., compound 14 or 22 of Examples 1 or 2);

L-2 second ligand for muscarinic receptor (e.g., compound 18 or 23 of Examples 1 or 2).

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-2 above and the second ligand and linker is selected from the following:

	L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
	L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
	L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
25	L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
	L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
	L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
	L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
	L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
30	L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
	L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
	L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
	L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
	L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
35	L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
	L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-

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	L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
	L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-
	L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-
	L-1/X-109-	L-1/X-110-	L-1/X-111-	L-1/X-112-	L-1/X-113-	L-1/X-114-
5	L-1/X-115-	L-1/X-116-	L-1/X-117-	L-1/X-118-	L-1/X-119-	L-1/X-120-
	L-1/X-121-	L-1/X-122-	L-1/X-123-	L-1/X-124-	L-1/X-125-	L-1/X-126-
	L-1/X-127-	L-1/X-128-	L-1/X-129-	L-1/X-130-	L-1/X-131-	L-1/X-132-
	L-1/X-133-	L-1/X-134-	L-1/X-135-	L-1/X-136-	L-1/X-137-	L-1/X-138-
	L-1/X-139-	L-1/X-140-	L-1/X-141-	L-1/X-142-	L-1/X-143-	L-1/X-144-
10	L-1/X-145-	L-1/X-146-	L-1/X-147-	L-1/X-148-	L-1/X-149-	L-1/X-150-
	L-1/X-151-	L-1/X-152-	L-1/X-153-	L-1/X-154-	L-1/X-155-	L-1/X-156-
	L-1/X-157-	L-1/X-158-	L-1/X-159-	L-1/X-160-	L-1/X-161-	L-1/X-162-
	L-1/X-163	L-1/X-164	L-1/X-165	L-1/X-166	L-1/X-167	L-1/X-168
	L-1/X-169	L-1/X-170	L-1/X-171	L-1/X-172		
15	L-1/X-173-	L-1/X-174-	L-1/X-175-	L-1/X-176-	L-1/X-177-	L-1/X-178-
	L-1/X-179-	L-1/X-180-	L-1/X-181-	L-1/X-182-	L-1/X-183-	L-1/X-184-
	L-1/X-185-	L-1/X-186-	L-1/X-187-	L-1/X-188-	L-1/X-189-	L-1/X-190-
	L-1/X-191-	L-1/X-192-	L-1/X-193-	L-1/X-194-	L-1/X-195-	L-1/X-196-
	L-1/X-197-	L-1/X-198-	L-1/X-199-	L-1/X-200-	L-1/X-201-	L-1/X-202-
20	L-1/X-203-	L-1/X-204-	L-1/X-205-	L-1/X-206-	L-1/X-207-	L-1/X-208-
	L-1/X-209-	L-1/X-210-	L-1/X-211-	L-1/X-212-	L-1/X-213-	L-1/X-214-
	L-1/X-215-	L-1/X-216-	L-1/X-217-	L-1/X-218-	L-1/X-219-	L-1/X-220-
	L-1/X-221-	L-1/X-222-	L-1/X-223-	L-1/X-224-	L-1/X-225-	L-1/X-226-
	L-1/X-227-	L-1/X-228-	L-1/X-229-	L-1/X-230-	L-1/X-231-	L-1/X-232-
25	L-1/X-233-	L-1/X-234-	L-1/X-235-	L-1/X-236-	L-1/X-237-	L-1/X-238-
	L-1/X-239-	L-1/X-240-	L-1/X-241-	L-1/X-242-	L-1/X-243-	L-1/X-244-
	L-1/X-245-	L-1/X-246-	L-1/X-247-	L-1/X-248-	L-1/X-249-	L-1/X-250-
	L-1/X-251-	L-1/X-252-	L-1/X-253-	L-1/X-254-	L-1/X-255-	L-1/X-256-
	L-1/X-257-	L-1/X-258-	L-1/X-259-	L-1/X-260-	L-1/X-261-	L-1/X-262-
30	L-1/X-263-	L-1/X-264-	L-1/X-265-	L-1/X-266-	L-1/X-267-	L-1/X-268-
	L-1/X-269-	L-1/X-270-	L-1/X-271-	L-1/X-272-	L-1/X-273-	L-1/X-274-
	L-1/X-275-	L-1/X-276-	L-1/X-277-	L-1/X-278-	L-1/X-279-	L-1/X-280-
	L-1/X-281-	L-1/X-282-	L-1/X-283-	L-1/X-284-	L-1/X-285-	L-1/X-286-
	L-1/X-287-	L-1/X-288-	L-1/X-289-	L-1/X-290-	L-1/X-291-	L-1/X-292-
35	L-1/X-293-	L-1/X-294-	L-1/X-295-	L-1/X-296-	L-1/X-297-	L-1/X-298-
	L-1/X-299-	L-1/X-300-	L-1/X-301-	L-1/X-302-	L-1/X-303-	L-1/X-304-
	L-1/X-305-	L-1/X-306-	L-1/X-307-	L-1/X-308-	L-1/X-309-	L-1/X-310-
	L-1/X-311-	L-1/X-312-	L-1/X-313-	L-1/X-314-	L-1/X-315-	L-1/X-316-
	L-1/X-317-	L-1/X-318-	L-1/X-319-	L-1/X-320-	L-1/X-321-	L-1/X-322-
40	L-1/X-323-	L-1/X-324-	L-1/X-325-	L-1/X-326-	L-1/X-327-	L-1/X-328-
	L-1/X-329-	L-1/X-330-	L-1/X-331-	L-1/X-332-	L-1/X-333-	L-1/X-334-
	L-1/X-335-	L-1/X-336-	L-1/X-337-	L-1/X-338-	L-1/X-339-	L-1/X-340-
	L-1/X-341-	L-1/X-342-	L-1/X-343-	L-1/X-344-	L-1/X-345-	L-1/X-346-
	L-1/X-347-	L-1/X-348-	L-1/X-349-	L-1/X-350-	L-1/X-351-	L-1/X-352-
45	L-1/X-353-	L-1/X-354-	L-1/X-355-	L-1/X-356-	L-1/X-357-	L-1/X-358-
	L-1/X-359-	L-1/X-360-	L-1/X-361-	L-1/X-362-	L-1/X-363-	L-1/X-364-

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	L-1/X-365-	L-1/X-366-	L-1/X-367-	L-1/X-368-	L-1/X-369-	L-1/X-370-
	L-1/X-371-	L-1/X-372-	L-1/X-373-	L-1/X-374-	L-1/X-375-	L-1/X-376-
	L-1/X-377-	L-1/X-378-	L-1/X-379-	L-1/X-380-	L-1/X-381-	L-1/X-382-
	L-1/X-383-	L-1/X-384-	L-1/X-385-	L-1/X-386-	L-1/X-387-	L-1/X-388-
5	L-1/X-389-	L-1/X-390-	L-1/X-391-	L-1/X-392-	L-1/X-393-	L-1/X-394-
	L-1/X-395-	L-1/X-396-	L-1/X-397-	L-1/X-398-	L-1/X-399-	L-1/X-400-
	L-1/X-401-	L-1/X-402-	L-1/X-403-	L-1/X-404-	L-1/X-405-	L-1/X-406-
	L-1/X-407-	L-1/X-408-	L-1/X-409-	L-1/X-410-	L-1/X-411-	L-1/X-412-
	L-1/X-413-	L-1/X-414-	L-1/X-415-	L-1/X-416-	L-1/X-417-	L-1/X-418-
10	L-2/X-1-	L-2/X-2-	L-2/X-3-	L-2/X-4-	L-2/X-5-	L-2/X-6-
	L-2/X-7-	L-2/X-8-	L-2/X-9-	L-2/X-10-	L-2/X-11-	L-2/X-12-
	L-2/X-13-	L-2/X-14-	L-2/X-15-	L-2/X-16-	L-2/X-17-	L-2/X-18-
	L-2/X-19-	L-2/X-20-	L-2/X-21-	L-2/X-22-	L-2/X-23-	L-2/X-24-
15	L-2/X-25-	L-2/X-26-	L-2/X-27-	L-2/X-28-	L-2/X-29-	L-2/X-30-
	L-2/X-31-	L-2/X-32-	L-2/X-33-	L-2/X-34-	L-2/X-35-	L-2/X-36-
	L-2/X-37-	L-2/X-38-	L-2/X-39-	L-2/X-40-	L-2/X-41-	L-2/X-42-
	L-2/X-43-	L-2/X-44-	L-2/X-45-	L-2/X-46-	L-2/X-47-	L-2/X-48-
	L-2/X-49-	L-2/X-50-	L-2/X-51-	L-2/X-52-	L-2/X-53-	L-2/X-54-
20	L-2/X-55-	L-2/X-56-	L-2/X-57-	L-2/X-58-	L-2/X-59-	L-2/X-60-
	L-2/X-61-	L-2/X-62-	L-2/X-63-	L-2/X-64-	L-2/X-65-	L-2/X-66-
	L-2/X-67-	L-2/X-68-	L-2/X-69-	L-2/X-70-	L-2/X-71-	L-2/X-72-
	L-2/X-73-	L-2/X-74-	L-2/X-75-	L-2/X-76-	L-2/X-77-	L-2/X-78-
	L-2/X-79-	L-2/X-80-	L-2/X-81-	L-2/X-82-	L-2/X-83-	L-2/X-84-
25	L-2/X-85-	L-2/X-86-	L-2/X-87-	L-2/X-88-	L-2/X-89-	L-2/X-90-
	L-2/X-91-	L-2/X-92-	L-2/X-93-	L-2/X-94-	L-2/X-95-	L-2/X-96-
	L-2/X-97-	L-2/X-98-	L-2/X-99-	L-2/X-100-	L-2/X-101-	L-2/X-102-
	L-2/X-103-	L-2/X-104-	L-2/X-105-	L-2/X-106-	L-2/X-107-	L-2/X-108-
	L-2/X-109-	L-2/X-110-	L-2/X-111-	L-2/X-112-	L-2/X-113-	L-2/X-114-
30	L-2/X-115-	L-2/X-116-	L-2/X-117-	L-2/X-118-	L-2/X-119-	L-2/X-120-
	L-2/X-121-	L-2/X-122-	L-2/X-123-	L-2/X-124-	L-2/X-125-	L-2/X-126-
	L-2/X-127-	L-2/X-128-	L-2/X-129-	L-2/X-130-	L-2/X-131-	L-2/X-132-
	L-2/X-133-	L-2/X-134-	L-2/X-135-	L-2/X-136-	L-2/X-137-	L-2/X-138-
	L-2/X-139-	L-2/X-140-	L-2/X-141-	L-2/X-142-	L-2/X-143-	L-2/X-144-
35	L-2/X-145-	L-2/X-146-	L-2/X-147-	L-2/X-148-	L-2/X-149-	L-2/X-150-
	L-2/X-151-	L-2/X-152-	L-2/X-153-	L-2/X-154-	L-2/X-155-	L-2/X-156-
	L-2/X-157-	L-2/X-158-	L-2/X-159-	L-2/X-160-	L-2/X-161-	L-2/X-162-
	L-2/X-163	L-2/X-164	L-2/X-165	L-2/X-166	L-2/X-167	L-2/X-168
	L-2/X-169	L-2/X-170	L-2/X-171	L-2/X-172		
40	L-2/X-173-	L-2/X-174-	L-2/X-175-	L-2/X-176-	L-2/X-177-	L-2/X-178-
	L-2/X-179-	L-2/X-180-	L-2/X-181-	L-2/X-182-	L-2/X-183-	L-2/X-184-
	L-2/X-185-	L-2/X-186-	L-2/X-187-	L-2/X-188-	L-2/X-189-	L-2/X-190-
	L-2/X-191-	L-2/X-192-	L-2/X-193-	L-2/X-194-	L-2/X-195-	L-2/X-196-
	L-2/X-197-	L-2/X-198-	L-2/X-199-	L-2/X-200-	L-2/X-201-	L-2/X-202-
45	L-2/X-203-	L-2/X-204-	L-2/X-205-	L-2/X-206-	L-2/X-207-	L-2/X-208-
	L-2/X-209-	L-2/X-210-	L-2/X-211-	L-2/X-212-	L-2/X-213-	L-2/X-214-

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	L-2/X-215-	L-2/X-216-	L-2/X-217-	L-2/X-218-	L-2/X-219-	L-2/X-220-
	L-2/X-221-	L-2/X-222-	L-2/X-223-	L-2/X-224-	L-2/X-225-	L-2/X-226-
	L-2/X-227-	L-2/X-228-	L-2/X-229-	L-2/X-230-	L-2/X-231-	L-2/X-232-
	L-2/X-233-	L-2/X-234-	L-2/X-235-	L-2/X-236-	L-2/X-237-	L-2/X-238-
5	L-2/X-239-	L-2/X-240-	L-2/X-241-	L-2/X-242-	L-2/X-243-	L-2/X-244-
	L-2/X-245-	L-2/X-246-	L-2/X-247-	L-2/X-248-	L-2/X-249-	L-2/X-250-
	L-2/X-251-	L-2/X-252-	L-2/X-253-	L-2/X-254-	L-2/X-255-	L-2/X-256-
	L-2/X-257-	L-2/X-258-	L-2/X-259-	L-2/X-260-	L-2/X-261-	L-2/X-262-
	L-2/X-263-	L-2/X-264-	L-2/X-265-	L-2/X-266-	L-2/X-267-	L-2/X-268-
10	L-2/X-269-	L-2/X-270-	L-2/X-271-	L-2/X-272-	L-2/X-273-	L-2/X-274-
	L-2/X-275-	L-2/X-276-	L-2/X-277-	L-2/X-278-	L-2/X-279-	L-2/X-280-
	L-2/X-281-	L-2/X-282-	L-2/X-283-	L-2/X-284-	L-2/X-285-	L-2/X-286-
	L-2/X-287-	L-2/X-288-	L-2/X-289-	L-2/X-290-	L-2/X-291-	L-2/X-292-
	L-2/X-293-	L-2/X-294-	L-2/X-295-	L-2/X-296-	L-2/X-297-	L-2/X-298-
15	L-2/X-299-	L-2/X-300-	L-2/X-301-	L-2/X-302-	L-2/X-303-	L-2/X-304-
	L-2/X-305-	L-2/X-306-	L-2/X-307-	L-2/X-308-	L-2/X-309-	L-2/X-310-
	L-2/X-311-	L-2/X-312-	L-2/X-313-	L-2/X-314-	L-2/X-315-	L-2/X-316-
	L-2/X-317-	L-2/X-318-	L-2/X-319-	L-2/X-320-	L-2/X-321-	L-2/X-322-
	L-2/X-323-	L-2/X-324-	L-2/X-325-	L-2/X-326-	L-2/X-327-	L-2/X-328-
20	L-2/X-329-	L-2/X-330-	L-2/X-331-	L-2/X-332-	L-2/X-333-	L-2/X-334-
	L-2/X-335-	L-2/X-336-	L-2/X-337-	L-2/X-338-	L-2/X-339-	L-2/X-340-
	L-2/X-341-	L-2/X-342-	L-2/X-343-	L-2/X-344-	L-2/X-345-	L-2/X-346-
	L-2/X-347-	L-2/X-348-	L-2/X-349-	L-2/X-350-	L-2/X-351-	L-2/X-352-
	L-2/X-353-	L-2/X-354-	L-2/X-355-	L-2/X-356-	L-2/X-357-	L-2/X-358-
25	L-2/X-359-	L-2/X-360-	L-2/X-361-	L-2/X-362-	L-2/X-363-	L-2/X-364-
	L-2/X-365-	L-2/X-366-	L-2/X-367-	L-2/X-368-	L-2/X-369-	L-2/X-370-
	L-2/X-371-	L-2/X-372-	L-2/X-373-	L-2/X-374-	L-2/X-375-	L-2/X-376-
	L-2/X-377-	L-2/X-378-	L-2/X-379-	L-2/X-380-	L-2/X-381-	L-2/X-382-
	L-2/X-383-	L-2/X-384-	L-2/X-385-	L-2/X-386-	L-2/X-387-	L-2/X-388-
30	L-2/X-389-	L-2/X-390-	L-2/X-391-	L-2/X-392-	L-2/X-393-	L-2/X-394-
	L-2/X-395-	L-2/X-396-	L-2/X-397-	L-2/X-398-	L-2/X-399-	L-2/X-400-
	L-2/X-401-	L-2/X-402-	L-2/X-403-	L-2/X-404-	L-2/X-405-	L-2/X-406-
	L-2/X-407-	L-2/X-408-	L-2/X-409-	L-2/X-410-	L-2/X-411-	L-2/X-412-
	L-2/X-413-	L-2/X-414-	L-2/X-415-	L-2/X-416-	L-2/X-417-	L-2/X-418-
35						

In addition to the combinatorial aspects of this invention, there is also provided an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor.

Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of

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ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is
5 conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric
10 compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which
15 elaborate upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said
20 molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times and more preferably from 2 to 50 times, even more preferably from 3 to 50 times and still more preferably
25 form 5 to 10 times.

This iterative process can employ collections of multimeric compounds which are prepared either by conventional sequential synthesis (a synthetic process involving a number of steps to provide a single compound which is then repeated with appropriate
30 alteration in use of reagents or process conditions to provide a second compound and wherein the repetition occurs n times to provide for n multimeric compounds) or by

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combinatorial synthesis to provide a multiplicity of compounds from a single synthetic pathway. In either event, in the first iteration, the ligands and linkers employed and the type and position of the reactive functional groups thereon are selected to provide a diverse collection of multimeric compounds thereby providing maximal information regarding those molecular constraints imparting multibinding properties. Subsequent iterations fine tune the constraints until a final "lead compound" is prepared.

Utility

The methods and libraries described herein are useful in determining multimeric compounds having multibinding properties. In turn, such multibinding compounds comprise two or more copies of ligands which treat disease conditions. Suitable ligands and disease conditions mediated thereby are illustrated above and multimeric compounds prepared by the methods and libraries described herein enable the discovery of new drug entities useful in the treatment of pathologic conditions in mammals, particularly humans.

The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this invention. Unless otherwise stated, all temperatures are in degrees Celsius.

EXAMPLES

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

DIPEA	=	diisopropylethylamine
eq.	=	equivalents
g	=	grams
h	=	hours
HPLC	=	high performance liquid chromatography
LC/MS	=	liquid chromatography/mass spectroscopy
mg	=	milligram
mL	=	milliliter
mmol	=	millimol
M	=	molarity
MS	=	mass spectroscopy
TFA	=	trifluoroacetic acid

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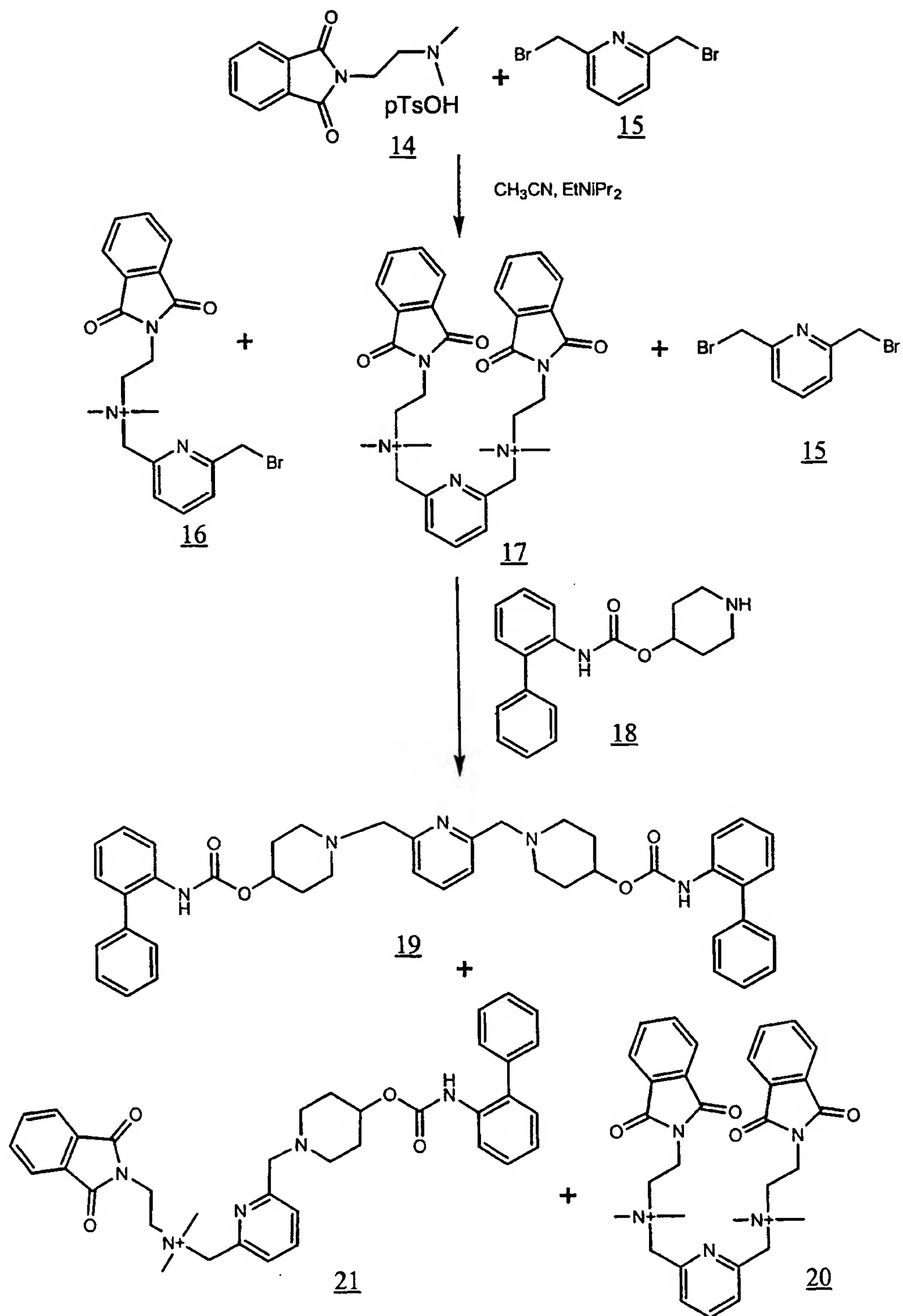
Examples 1 and 2 illustrate combinatorial methods for preparing libraries of multimeric compounds which can be evaluated for possessing properties consistent with multibinding compounds. In Examples 1 and 2, the library utilizes two different ligands [L-1 and L-2] for the muscarinic receptor and a single linker, X. In each example, combinatorial chemistry produces a library of 3 members which, for ease of identification are represented as L-1/X/L-1; L-2/X/L-1; and L-2/X/L-2.

Example 1

Synthesis of a Library of Multimeric Compounds via Combinatorial Chemistry

This example illustrates combinatorial synthesis using sequential protocols. The first ligand is represented by compound 14; the second ligand is represented by compound 18; and the linker is represented by compound 15. The combinatorial reaction scheme is depicted below:

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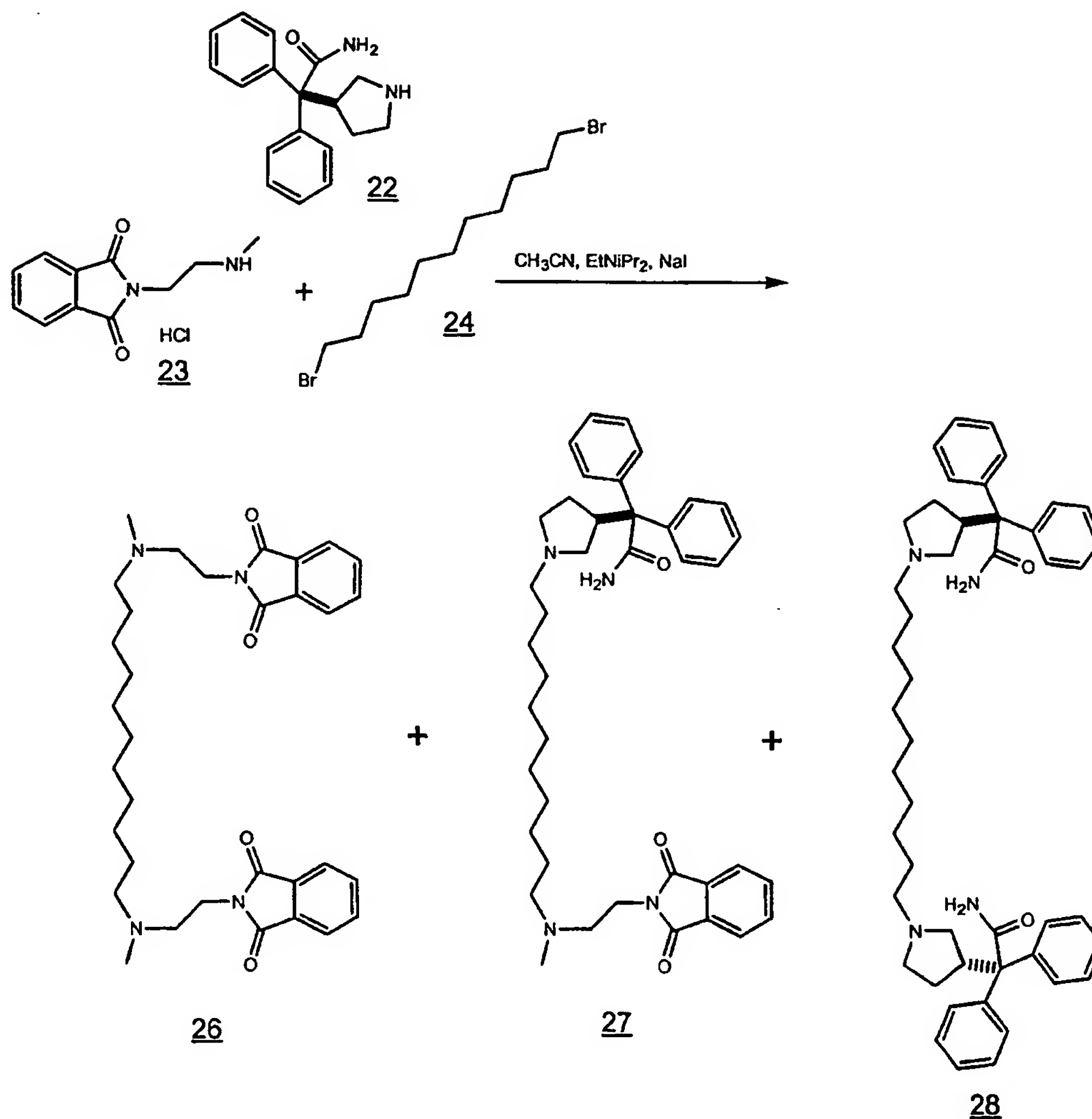
Specifically, an aliquot (0.22 mL) of a solution prepared from N-[2-dimethylamino)-ethyl]phthalimido 14 (1.25 g, 3.2 mmol) and EtNiPr₂ (0.79 mL) dissolved in enough anhydrous acetonitrile to bring the total volume up 6.4 mL was added to a 1 dram vial charged with 2,6-bis(bromomethyl)pyridine (Aldrich, 26.5 mg, 0.10 mmol) in 0.22 mL of acetonitrile). The vial was closed with a Teflon sealed cap and the placed in a 72 °C heating block for a 24 h to give a mixture of compounds 15, 16, and 17. After cooling to room temperature, 4-piperidyl-N-(2-biphenyl)-carbamate 18 (0.33 mL) (prepared by dissolving 2.96 g of 18 in anhydrous DMF to produce a total volume of 33 mL) was added and the vial is resealed and heated overnight at 72 °C in a heating block. The mixture was cooled, quenched with 5% TFA/water (0.30 mL), diluted with acetonitrile and water, filtered, and purified using preperative LC/MS [Zeng, L; Kassel, D. B. *Anal. Chem.* 1998, 70, 4380-4388 and references therein] to provide the individual components. Quality and identity of the collected fractions was verified using analytical HPLC and electrospray MS.

Example 2

Synthesis of a Library of Multimeric Compounds via Combinatorial Chemistry

This example illustrates combinatoral synthesis using concurrent protocols. The first ligand is represented by compound 22; the second ligand is represented by compound 23; and the linker is represented by compound 24. The combinatorial reaction scheme is depicted below:

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N-(2-Methylaminethyl)phthalimido **23** (0.20 mL, of a 0.5 M solution, 0.10 mmol) (prepared by dissolving 168 mg of N-(2-methylaminethyl)phthalimido in, DIPEA (0.18 mL) and enough anhydrous acetonitrile to bring the solution to a total volume of 1.4 mL), and a solution of compound **22** (0.167 mL) (prepared by dissolving 673 mg of **22** in enough anhydrous acetonitrile to bring the total volume to 4 mL), and NaI (0.20 mL of a 1 M solution in anhydrous acetonitrile) were combined in a 1 dram vial charged with 1,11-dibromoundecane (0.10 mmol). The vial was closed with a Teflon sealed cap and the placed in a 72 °C heating block for a 21 h. The mixture was cooled, quenched with

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5% TFA/water (0.30 mL), diluted with acetonitrile and water, filtered, and purified using preparative LC/MS [Zeng, L; Kassel, D. B. *Anal. Chem.* 1998, 70, 4380-4388 and references therein] to provide the individual components 25-28. Quality and identity of the collected fractions was verified using analytical HPLC and electrospray MS.

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Each of the isolated members from the above combinatorial libraries can be assayed to determine which, if any, of these multimeric compounds possess properties consistent with multibinding compounds. Key molecular constraints consistent with such properties can then be determined and elaborated upon in a next library iteration. The process can be repeated as necessary to determine multimeric compounds with high degrees of activity consistent with multibinding properties.

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WHAT IS CLAIMED IS:

1. A method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

- 5 (a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;
- 10 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and
- 15 (d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

2. A method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

- 20 (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;
- (b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the
- 25 reactive functional groups of the ligand;
- (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of
- 30 said ligands; and
- (d) assaying the multimeric ligand compounds produced in the library

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prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties.

3. The method according to Claim 1 or 2 wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

4. The method according to Claim 3 wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

5. The method according to Claim 4 wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

6. The method according to Claim 5 wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

7. The method according to Claim 1 or 2 wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

8. The method according to Claim 7 wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

9. The method according to Claim 1 or Claim 2 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

10. The method according to Claim 9 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

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11. The method according to Claim 10 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

12. The method according to Claim 1 or 2 wherein the ligand or mixture of
5 ligands is selected to have reactive functionality at different sites on said ligands.

13. The method according to Claim 12 wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones,
10 aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

14. The method according to Claim 1 or Claim 2 wherein the multimeric
15 ligand compound library comprises homomeric ligand compounds.

15. The method according to Claim 1 or Claim 2 wherein the multimeric
ligand compound library comprises heteromeric ligand compounds.

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16. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

25 (b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the
30 library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said

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ligands.

17. A library of multimeric ligand compounds which may possess multivalent properties which library is prepared by the method comprising:

5 (a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

10 (c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

15 18. The library according to Claim 16 or Claim 17 wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic
20 linkers.

19. The library according to Claim 18 wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

25 20. The library according to Claim 19 wherein the linkers are selected to have different linker lengths ranging from about 2 to 100Å.

21. The library according to Claim 16 or 17 wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

30 22. The library according to Claim 21 wherein said reactive functionality is

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selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

23. The library according to Claim 16 or Claim 17 wherein the multimeric ligand compound library comprises homomeric ligand compounds.

24. The library according to Claim 16 or Claim 17 wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

25. An iterative method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which

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elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

26. The method according to Claim 25 wherein steps (e) and (f) are repeated from 2-50 times.

27. The method according to Claim 15 wherein steps (e) and (f) are repeated from 5-50 times.

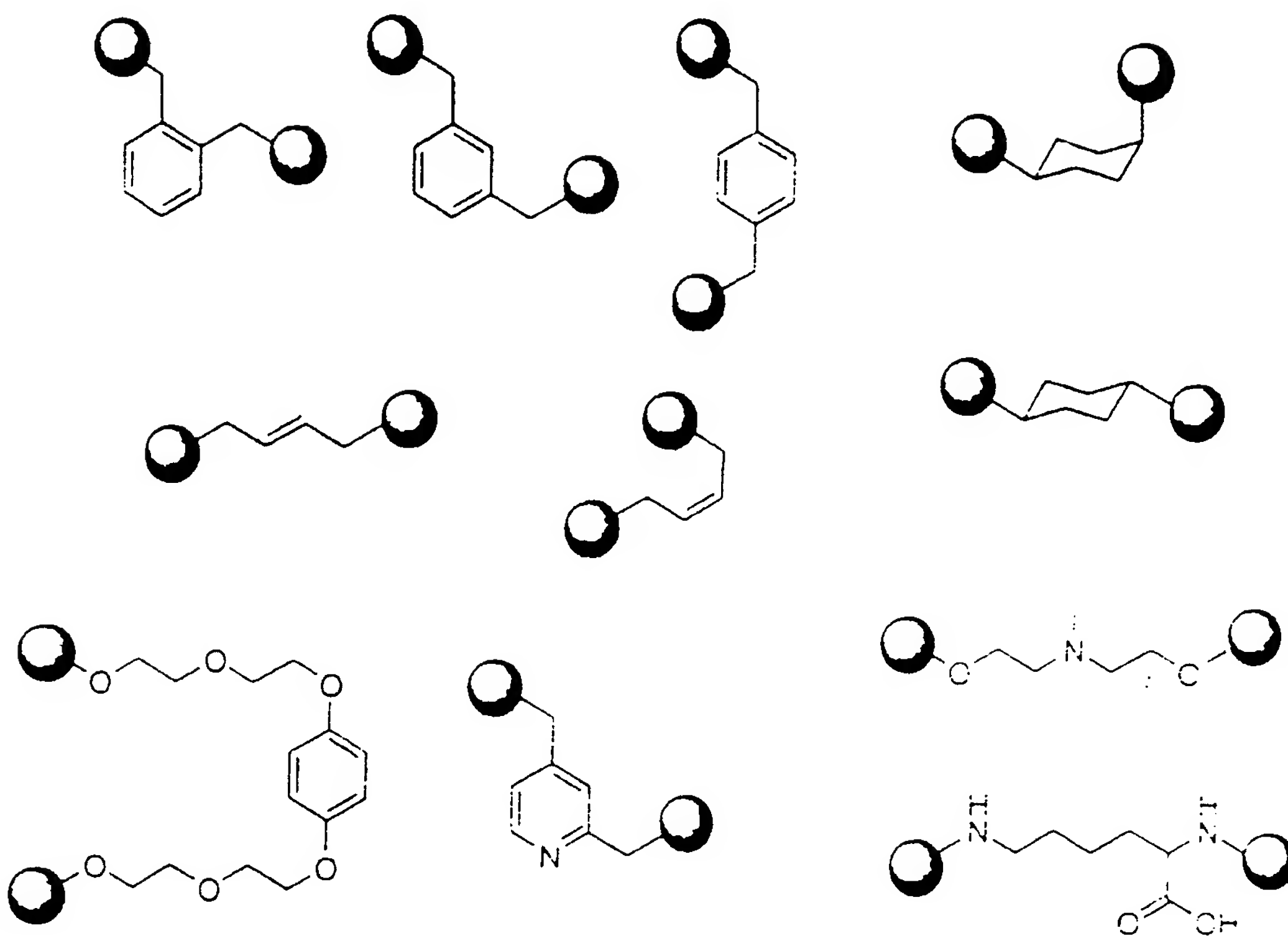
Examples of dimeric display

FIGURE 1

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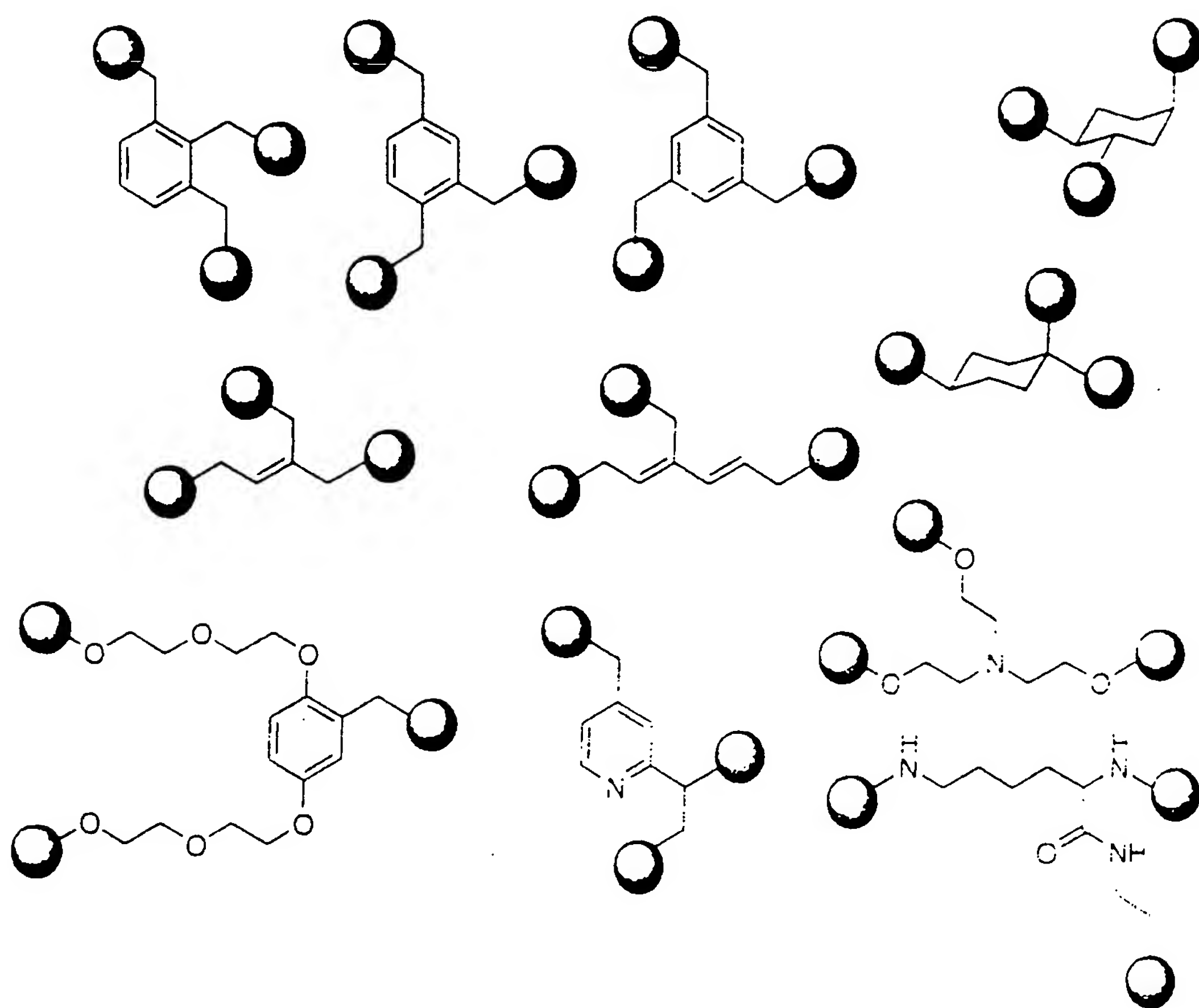
Examples of trimeric display

FIGURE 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11788

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501/518; 530/345, 389.1, 402, 807

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN (CAPLUS, BIOSIS, SCISEARCH, MEDLINE)

Search Terms: multivalent, multibinding, linker, covalent, lignad, combinatorial, library

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	LIANG et al. Parallel Synthesis and Screening of a Solid Phase Carbohydrate Library. Science. 29 November 1996, Vol. 274, pages 1520-1522. See entire article.	17, 18, 24 --- 1-16, 19-23, 25-27
X --- Y	COLE et al. Discovery of Chiral Catalysts through Ligand Diversity: Ti-Catalyzed Enantioselective Addition of TMSCN to meso Epoxides. Angew. Chem. Intl. Ed. Engl. 1996, Vol. 35, No. 15, pages 1668-1671. See pages 1669-1670, Figure 1 and Scheme 2.	16-18, 24 --- 1-15, 17-23, 25-27
Y	MENGER et al. Phosphatase Catalysis Developed via Combinatorial Organic Chemistry. J. Org. Chem. 1995, Vol. 60, pages 6666-6667. See entire article.	1-27



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

26 AUGUST 1999

Date of mailing of the international search report

26 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
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Washington, D.C. 20231

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JOYCE BRIDGERS
PARALEGAL SPECIALIST
CHEMICAL MATRIX

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11788

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/35195 A1 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 25 September 1997 (25.09.97), see page 3 lines 17-32, page 4 lines 1-18, page 7 lines 26-34, page 8 lines 1-5 and claims 13, 35 & 36.	1-27
Y	DAVIS et al. Drug Leads from Combinatorial Phosphodiester Libraries. J. Med. Chem. 27 October 1995, Vol. 38, No. 22, pages 4363-4366. See entire article.	1-27
Y	GARDINER, J. M. 'The Therapeutic Potential of Synthetic Multivalent Carbohydrates'. In: Expert Opin. Invest Drugs. March 1998, Vol. 7, No. 3, pages 405-411. See entire article.	1-27
Y	SHUKER et al. Discovering High-Affinity Lignads for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11788

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☒

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11788

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501/518; 530/345, 389.1, 402, 807

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s) 1-14, drawn to a method for identifying multimeric ligand compounds.

Group II, claim(s) 15-24, drawn to a library of multimeric ligand compounds.

Group III, claim(s) 25-27, drawn to an iterative method for identifying multimeric ligand compounds.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature that links all of the claims is the multimeric ligand compound library. These libraries are known in the art.

For example, Cole et al (Angew. Chem. Int. Ed. Engl., 1996, Vol. 35, No. 15, pp. 1668-1671) teaches "diverse peptide-based structures" that can be collectively screened to search for chiral catalysts (see page 1669, 1st column). These ligands possess at least three sites for binding, as shown in structure 3 (2 hydroxy groups and the nitrogen). Figure 1 of Cole et al (page 1670) shows the variation of the ligand components. The middle amino acid component (AA2) is a linker, with the amino and acid ends comprising two functional groups having complementary reactivity to the other portions of the ligand. Cole et al shows using a library of linkers (choices for AA2 in Figure 1). The other portions are varied as shown in the figure (other amino acid segment and aldehyde segments a-m) and form covalent linkages to AA2 by reaction with the complementary functional groups (see Scheme 2, page 1669).

Additionally, Menger et al (J. Org. Chem., 1995, Vol. 60, pp. 6666-6667) teaches a multimeric ligand compound library comprising a single linker molecule. The linker is polyallylamine, which has a multitude of amino groups that are complementary to the library of ligands having a reactive carboxylic acid functionality. The library of ligands are covalently attached to the linker polymer; this is shown in Figure 1 (page 6666).

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